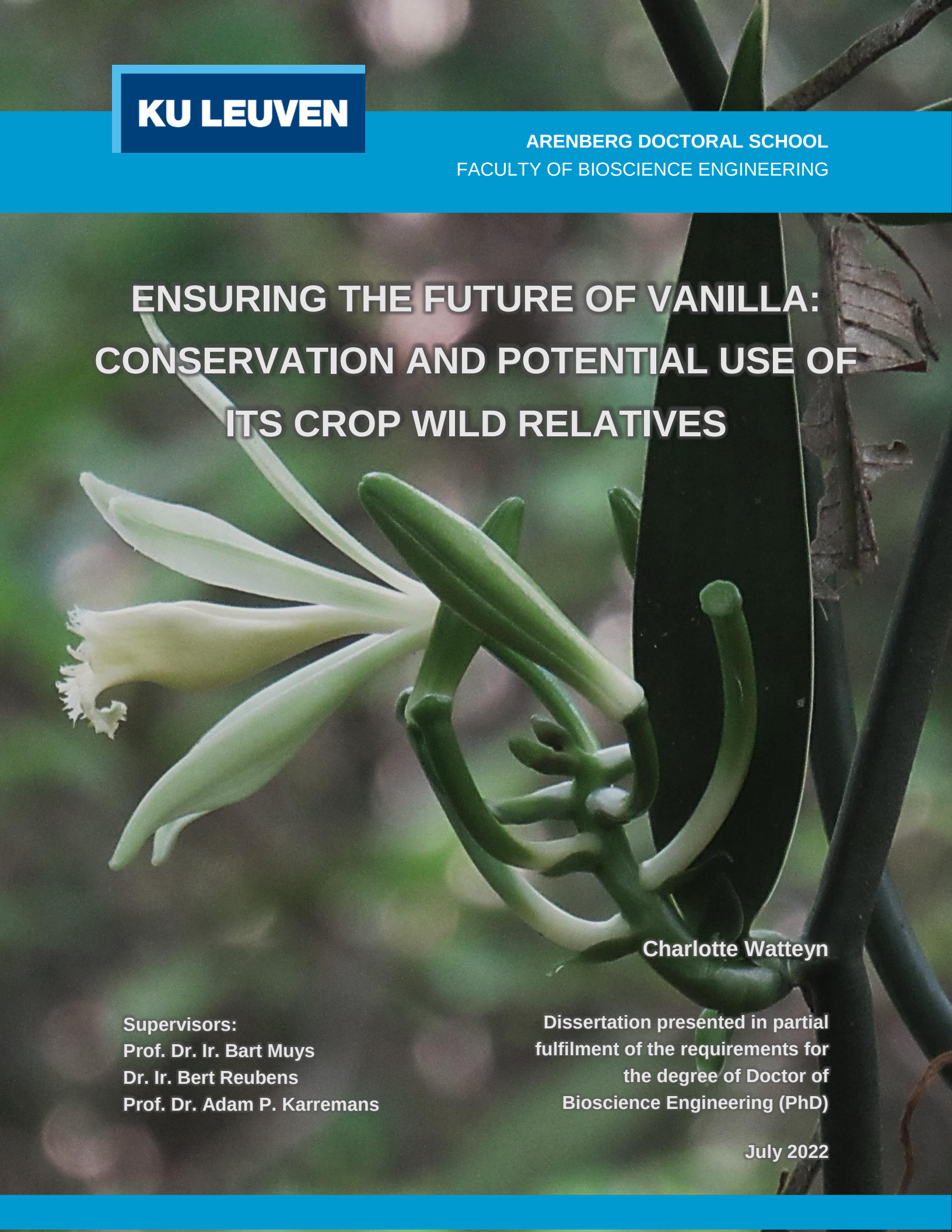

KU LEUVEN

ARENBERG DOCTORAL SCHOOL
FACULTY OF BIOSCIENCE ENGINEERING

ENSURING THE FUTURE OF VANILLA: CONSERVATION AND POTENTIAL USE OF ITS CROP WILD RELATIVES

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Dissertation presented in partial fulfilment of the requirements for the degree of Doctor of Bioscience Engineering (PhD)

July 2022

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- **Leave the road, take the trails -**

Pythagoras

TABLE OF CONTENTS

Acknowledgments ..... **iii**

Summary ..... **vii**

Samenvatting ..... **xi**

Resumen ..... **xv**

List of tables ..... **xix**

List of figures ..... **xxi**

Abbreviations ..... **xxvii**

Chapter 1. Introduction **1**

1.1 Context and problem statement 5
1.1.1. Food production and its environmental and social impact 5
1.1.2. Let's spice it up: The vanilla sector as an example 6
1.2. Back to the wild with crop wild relatives 8
1.2.1. Present state of knowledge on crop wild relatives 8
1.2.2. *Vanilla* crop wild relatives 11
1.3. Towards a multifunctional land use strategy for the vanilla crop 12
1.3.1. Land sparing versus land sharing 12
1.3.2. A combined land sparing/sharing approach for *Vanilla* conservation and cultivation 14
1.4. Supply chain management 16
1.4.1. Issues with global food supply 16
1.4.2. Driving sustainability within the vanilla supply chain 17
1.5. Aims, objectives and thesis outline 20
1.4.1. Aims and objectives 20
1.4.2. Thesis outline 21
1.6. Study area 23

Chapter 2. Vanilla distribution modeling for conservation and sustainable cultivation in a joint land sparing/sharing approach **25**

2.1. Summary 29
2.2. Introduction 31
2.3. Materials and methods 34
2.3.1. Study area 34
2.3.2. Study species 34
2.3.3. Species distribution modelling 35
2.4. Results 39
2.4.1. Species distribution modelling 39
2.4.2. Maps for priority conservation and sustainable cultivation of *Vanilla* CWRs 41
2.5. Discussion 43
2.5.1. Vanilla distribution maps 43
2.5.2. Joint land sharing / sparing concept (SPASHA) 44
2.5.3. Implementation in land use policy 47
2.6. Conclusions 47

Chapter 3. Trick or treat? Pollinator attraction in *Vanilla pompona* (Orchidaceae) 49

3.1. Summary 53
3.2. Introduction 55
3.3. Materials and methods 56
3.3.1. Study area and species 56
3.3.2. Floral visitors and their behaviour 57
3.3.3. Pollinator identification and pollination success 58
3.3.4. Floral rewards 58
3.3.5. Flower and floral visitor morphology 60
3.4. Results 61
3.4.1. Floral visitors and their behaviour 61
3.4.2. Pollinator identification and pollination success 62
3.4.3. Floral rewards 62
3.4.4. Flower and floral visitor morphology 64
3.5. Discussion 64
3.5.1. The dual pollinator attraction hypothesis 65
3.5.2. Morphological goodness-of-fit for pollen removal by *Eulaema* 66
3.6. Conclusions 67

Chapter 4. Sweet as *Vanilla hartii*: Evidence for a nectar-rewarding pollination mechanism in *Vanilla* (Orchidaceae) flowers 69

4.1. Summary 73
4.2. Introduction 75
4.3. Materials and methods 77
4.3.1. Study area and species 77
4.3.2. Floral rewards 78
4.3.3. Floral visitors and their behaviour: identifying potential pollinators 80
4.3.4. Pollination success 81
4.4. Results 81
4.4.1. Floral rewards 81
4.4.2. Floral visitors and their behaviour: identifying potential pollinators 82
4.4.3. Pollination success 84
4.5. Discussion 84
4.5.1. Nectar-rewarding pollination mechanism 84
4.5.2. Floral visitors and potential pollinators 85
4.5.3. Mapping vanilla pollination strategies 86
4.6. Concluding remarks on the pollination of fragrant *Vanilla* species 88

Chapter 5. Insights on early-growth performance and market potential of *Vanilla* crop wild relatives 91

5.1. Summary 95
5.2. Introduction 97
5.3. Materials and methods 99
5.3.1. Study area and species 99
5.3.2. Experimental design 100
5.3.3. Data collection 102
5.3.2. Data analysis 104
5.4. Results 106
5.4.1. Exploration of vanilla plant performance 106

5.4.2. Vanilla plant performance models 107
5.4.3. Aromatic potential of vanilla pods 116
5.5. Discussion 119
5.5.1. Vanilla plant performance: Survival, vitality, growth and earliness 119
5.5.2. Aromatic potential for market integration 122
5.6. Conclusions and next steps 123

Chapter 6. Exploring farmer preferences towards innovations in the vanilla supply chain 125

6.1. Summary 129
6.2. Introduction 131
6.3. Materials and methods 134
6.3.1. Study area 134
6.3.2. Design of the choice experiment 134
6.3.3. Hypotheses 137
6.3.4. Data collection 137
6.3.5. Econometric models 139
6.4. Results 139
6.4.1. Descriptive statistics of the respondents 139
6.4.2. Mixed logit model 140
6.4.3. Latent class model 140
6.5. Discussion 146
6.5.1. Farmer preferences regarding vanilla cultivation 146
6.5.2. Location of potential vanilla farmers within the landscape 147
6.5.3. Proposition for innovation in vanilla cultivation 148
6.5.4. Limitations of discrete choice experiments 150
6.6. Conclusions 150

Chapter 7. Discussion and conclusions 153

7.1. Conservation and cultivation of *Vanilla* CWRs 157
7.1.1. Theoretical development of a case-specific *Vanilla* SPASHA approach 157
7.1.2. Natural pollinators and their potential integration within vanilla cultivation 160
7.1.3. Cultivation potential of *Vanilla* CWRs within the context of SPASHA 164
7.1.4. Perceptions on innovations within the vanilla supply chain 172
7.1.5. From theory to practice 175
7.2. Conclusions and future research paths 176
7.1.4. Main conclusions 176
7.1.5. Limitations and further research 177

References 183

Appendices 215

List of publications 241



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SUMMARY

The vanilla sector faces several challenges that negatively affect its stable supply. Domestication bottlenecks, vegetative propagation, and manual pollination instigated a strong genetic erosion in the crop species *Vanilla planifolia*. Moreover, practices involving intense monocultures and supply chains with asymmetrical revenue partition further exacerbate the vulnerability of this sector. The Neotropics host several *Vanilla* crop wild relatives (CWRs). Alike the closely related *V. planifola*, *Vanilla* CWRs produce fragrant fruits, better known as beans or pods. Furthermore, they hold untapped diversity for potential use in crop improvement and breeding, yet, are threatened by extinction. There is an urgent need to protect these *Vanilla* species, and simultaneously evaluate their possible contribution to counteract the abovementioned production problems. This PhD thesis aimed at providing initial insights into the use of *Vanilla* CWRs within an approach that endeavors a joint effort of (a) the conservation of the natural habitat of *Vanilla* CWRs and their associated pollen and seed dispersers, and (b) the sustainable cultivation of the vanilla spice in agroforestry systems surrounding these protected areas, promoting the integration of promising *Vanilla* CWRs and natural pollinators. We define this as the *Vanilla* land sparing/sharing (SPASHA) approach, whereby the produced vanilla beans are ideally traded via direct supply chains that involve organized farmer groups and responsible businesses. We conducted an interdisciplinary study to explore the application potential of the proposed *Vanilla* SPASHA approach within our study region Área de Conservación Osa (ACOSA) – a biodiversity hotspot in southwest Costa Rica holding several *Vanilla* CWR populations. As such, this PhD implemented four work packages to acquire information on the distribution patterns, pollination mechanisms, and cultivation potential of *Vanilla* CWR's, as well as on the perceptions of the vanilla supply chain members towards the proposed innovations.

In the first work package (Chapter 2), we applied species distribution modelling to provide spatially explicit recommendations for the implementation of the *Vanilla* SPASHA approach in ACOSA. The maps, modelled at the extent of Costa Rica, showed interspecific variation in suitability, most likely influenced by species-specific functional plant traits or certain biotic interactions. ACOSA proved highly suitable for the four modelled CWRs, and suitability maps were overlaid with land use maps to define (a) low- and high-priority conservation areas, i.e. forested areas respectively with or without protection status, both with high *Vanilla* suitability, and (b) potential cultivation areas, i.e. fallow and degraded lands that could be reforested with the integration of promising *Vanilla* CWRs, or existing agricultural lands that could be transformed to agroforestry systems with vanilla as an (additional) cash crop. In this context, cultivation areas may serve as biological

corridors between the protected areas, encouraging the exchange of ecosystem services between forest and farmland. The spatially explicit recommendations resulting from this study fit within the scope of the National Bio-Corridor program that seeks to develop sustainable land use practices within the corridor network.

In the second work package (Chapters 3 and 4), we assessed the potential integration of natural pollinators within vanilla cultivation systems, as an alternative to the widely applied manual pollination technique. We identified pollinators and pollination mechanisms, examined pollinator habitat requirements, and evaluated natural fruit set. We found that *Vanilla pompona* is pollinated by *Eulaema cingulata*, and displays a dual pollinator attraction mechanism that combines floral fragrance rewards with food deception to induce pollen removal. *Vanilla hartii* rewards its floral visitors with sucrose-rich nectar, and is pollinated by *Euglossa* species that morphologically fit with the flowers. Combining our results with an in-depth literature review, we presume that all the Neotropical fragrant *Vanilla* CWRs are pollinated by Euglossini, with the latter demonstrating specific habitat requirements. Overall, the low natural fruit set, even of nectar-rewarding *Vanilla* species, favours the continuation of manual over natural pollination. Yet, we highly encourage the development of agro-ecological landscapes that consist of Euglossini-friendly vanilla cultivation systems surrounding Euglossini refuges (i.e. intact forests), whereby vanilla beans produced in these landscapes could be certified and sold at specialty markets.

In the third work package (Chapter 5), we obtained insights into the cultivation potential of the four modelled *Vanilla* CWRs. We established experimental field sites in both forested lands and existing agro-systems (i.e. cacao plantations) available in ACOSA, and developed models that predict the measured plant performance traits with *Vanilla* species (the four CWRs, a commercially-used hybrid, and the crop species *V. planifolia*), land use system, and several environmental variables. The hybrid and CWRs outperformed *V. planifolia* in terms of vitality, whereas the latter and the hybrid had the highest growth rate. The performance of each species, however, depended on land use system and light intensity, enabling us to identify species-specific preferences. Disease-suppressive and growth-promoting bacteria positively affected vanilla plant survival, vitality, and growth. Moreover, we observed significant effects of certain soil chemical variables and functional tutor tree traits on vanilla plant performance – emphasizing the important interactions between the vanilla orchid and its environment. Additionally, the quantified aromatic potential of the beans of the different *Vanilla* species demonstrates possibilities for market diversification. Our results demonstrate potential for the inclusion of *Vanilla* CWRs in cultivation systems that mimic their natural habitat. Yet, an evaluation of the feasibility (e.g. labour,

production, revenues) of these systems within the proposed SPASHA approach is highly required, alongside further research on the spatial arrangement and associated management of the different *Vanilla* species and the other, additional crops (e.g. cacao).

In the last work package (Chapter 6), we carried out a discrete choice experiment with 186 farmers living in ACOSA, to better understand the perspectives of the supply chain members towards innovations within the vanilla sector. Combining our results with a study on market preferences, we see great value in the development of a supply chain between (a) farmers willing to form part of a vanilla cooperative and interested in agro-ecological practices involving *Vanilla* CWRs, natural pollinators, and agroforestry, and (b) responsible businesses seeking sustainable vanilla sourcing and market diversification. In this context, farmer cooperatives will most likely play a central role in capacity building, farmer organization, and the provision of market outlets.

In conclusion, we provide insights into possible measures that could contribute to the mitigation of the identified production and socio-economic problems within the vanilla sector; by probing the potential of a strategy that combines the conservation and sustainable cultivation of *Vanilla* CWRs. We combined the data acquired from the four work packages and developed a practical scheme for the implementation of the case-specific *Vanilla* SPASHA approach in ACOSA – an approach that coincides with the national land use policies for this biodiversity hotspot as well as the perceptions of a large part of the questioned farmers. Yet, further evaluation of this approach in terms of production feasibility, biodiversity conservation, and market integration of new vanilla varieties is highly required.

SAMENVATTING

De vanillesector wordt geconfronteerd met verschillende uitdagingen die een continue voorraad aan vanillebonen verhinderen. Gewasdomesticatie, vegetatieve propagatie, en manuele bestuiving hebben geleid tot een sterke genetische erosie van het *Vanilla planifolia* gewas. Daarenboven verhogen intensief beheerde vanillemonoculturen en een vanilletoeleveringsketen gekenmerkt door asymmetrische opbrengstenverdeling, de kwetsbaarheid van deze sector nog meer. De Neotropen herbergen een ruim aanbod aan wilde aromatische vanillesoorten (CWR's). Net als de nauw verwante *V. planifolia*, produceren deze *Vanilla* CWR's aromatische bonen. Ze beschikken bovendien over een ongerepte genetische diversiteit die gebruikt kan worden voor gewasverbetering- en veredeling. *Vanilla* CWR's worden echter met uitsterven bedreigd. Het is als zodanig hoognodig om deze CWR's te beschermen, en om hun mogelijke bijdrage tot het oplossen van bovengenoemde productieproblemen te evalueren. Het doel van deze doctoraatsthesis was om enkele eerste inzichten te verschaffen in het potentieel gebruik van deze *Vanilla* CWR's, en dit gekaderd binnen een aanpak dat simultaan het volgende nastreeft: (a) de bescherming van de natuurlijke habitatten van *Vanilla* CWR's en hun stuifmeel- en zaadverspreiders, en (b) een duurzame vanilleteelt, met integratie van veelbelovende *Vanilla* CWR's en hun bestuivers, en dit in agroforestry systemen rondom bovengenoemde beschermde gebieden. We definiëren dit als de *Vanilla* SPASHA (*joint land sparing/sharing*) aanpak, waarbij de geproduceerde vanillebonen idealiter verhandeld worden via directe ketens tussen georganiseerde boeren en verantwoordelijke kooplui. Om de mogelijke toepassing van het *Vanilla* SPASHA concept in ons studiegebied Área de Conservación Osa (ACOSA) – een biodiversiteitshotspot in het zuidwesten van Costa Rica waar verscheidene wilde vanillepoplaties voorkomen – na te gaan, voerden we een interdisciplinair onderzoek uit. Er werden als dusdanig vier werkpakketten geformuleerd om informatie te verzamelen omtrent de ruimtelijke distributiepatronen, de bestuivingsmechanismen, en het teeltpotentieel van *Vanilla* CWR's, alsook de percepties van enkele sleutelactoren in de vanilletoeleveringsketen.

Om uitspraken te kunnen doen over een mogelijke implementatie van dit SPASHA concept in ons studiegebied ACOSA, voerden we in het eerste werkpakket (Hoofdstuk 2) twee een soortendistributiemodellering uit. De kaarten, gemodelleerd op de schaal van Costa Rica, toonden soortspecifieke habitatgeschiktheid aan dat hoogstwaarschijnlijk beïnvloed wordt door functionele plantenkarakteristieken en biotische interacties. ACOSA bleek geschikt te zijn voor de vier gemodelleerde CWR's, en we combineerden de kaarten met regionale landgebruikskaarten om (a) gebieden met lage en hoge beschermingsprioriteit te definiëren, respectievelijk beboste

gebieden met of zonder beschermingsstatus, en beide met hoge habitatgeschiktheid voor de gemodelleerde vanillesoorten, en (b) potentiële teeltgebieden, met inbegrip van braakliggende of gedegradeerde terreinen die herbebost kunnen worden met integratie van veelbelovende CWR's, of bestaande landbouwgronden die omgevormd kunnen worden tot agroforestry systemen met vanille als hoogwaardig handelsgewas. De vanilleteeltgebieden kunnen in deze context optreden als biologische corridors tussen de beschermde gebieden, waarbij de uitwisseling van bepaalde ecosysteemdiensten tussen bos en landbouwgrond gestimuleerd wordt. Onze ruimtelijk-expliciete aanbevelingen passen in het kader van het Costa Ricaans Bio-Corridor programma dat als doel heeft om duurzame activiteiten te promoten binnen het corridornetwerk.

In het derde werkpakket (Hoofdstukken 3 en 4) evalueerden we de potentiële integratie van natuurlijke bestuivers binnen vanilleteeltsystemen, als alternatief voor de manuele bestuivingstechniek. We identificeerden bestuivers en bestuivingsmechanismen, onderzochten de habitatvereisten van de bestuivers, en evalueerden de natuurlijke vruchtzetting. *Vanilla pompona* wordt bestoven door *Eulaema cingulata*, en vertoont een tweevoudig mechanisme om bestuivers aan te trekken, waarbij de productie van een sterke bloemengeur wordt gecombineerd met bestuiving door voedselmisleiding. *Vanilla hartii* beloont haar bloemenbezoekers met sucrose-rijke nectar, en wordt bestoven door *Euglossa* bijen die een morfologische fit met de bloem vertonen. Door onze resultaten te combineren met een diepgaande literatuurstudie, veronderstellen we dat alle Neotropische, aromatische *Vanilla* CWR's bestoven worden door Euglossini, en dat deze laatste specifieke habitatvereisten vertonen. We concluderen echter dat door de beperkte natuurlijke vruchtzetting, zelfs van de nectar-belonende vanillesoorten, het voor de vanilleboeren voordeliger blijft om manueel de vanillebloemen te bestuiven. Toch moedigen wij ten zeerste de creatie van een agro-ecologisch landschap aan, dat bestaat uit Euglossini-vriendelijke vanilleteeltsystemen rondom intacte bossen, om zo de uitwisseling van bestuivers te stimuleren. Vanillebonen die geproduceerd worden in deze landschappen, kunnen eventueel gecertificeerd en gecommercialiseerd worden via speciale toeleveringsketens.

In het derde werkpakket (Hoofdstuk 5) trachtten we om initiële inzichten te verwerven in het teeltpotentieel van de vier gemodelleerde *Vanilla* CWR's. We maakten experimentele proefvelden aan in zowel beboste gebieden als bestaande agro-systemen (cacaoplantages) van ACOSA, en ontwikkelden modellen die de opgemeten variabelen inzake plantenvitaliteit en -groei linken met vanillesoort (de vier CWR's, een commercieel gebruikte hybride, en *V. planifolia*), landgebruik, en verscheidene omgevingsvariabelen. De hybride en de CWR's presteerden beter dan *V. planifolia* inzake plantenvitaliteit, terwijl deze laatste en de hybride de sneltste groei vertoonden. De

prestaties van elke vanillesoort waren echter gecorreleerd met landgebruik en lichtintensiteit, waardoor we soortspecifieke teeltvoorkeuren konden identificeren. Ziekte-onderdrukkende en groeibevorderende bacteriën hadden een positieve impact op de overleving, vitaliteit en groei van de planten. We observeerden ook significante effecten van zowel chemische bodemvariabelen als bepaalde functionele eigenschappen van de vanillesteunbomen – wat de belangrijke interacties tussen vanilleorchideeën en hun omgeving benadrukt. Bovendien kwantificeerden we het aromatisch potentieel van de vanillebonen van de verschillende soorten, en zien we mogelijkheden voor marktdiversificatie. Onze resultaten tonen aan dat *Vanilla* CWR's potentieel vertonen om opgenomen te worden in teeltsystemen die hun natuurlijke habitat nabootsen. Bijkomend onderzoek inzake de haalbaarheid (bv. arbeid, productie, opbrengst) en de ruimtelijke gewasindeling is echter noodzakelijk.

In het laatste werkpakket (Hoofdstuk 6) voerden we een discreet keuze-experiment uit met 186 boeren die in ACOSA wonen, met als doel om de perspectieven van de verschillende actoren van de vanilletoeleveringsketen ten opzichte van vooropgestelde innovaties beter te begrijpen. Door onze resultaten te combineren met een studie omtrent voorkeuren in vanillevermarkting, zien we potentieel in de ontwikkeling van een innovatieve toeleveringsketen tussen (a) coöperatieve boeren die geïnteresseerd zijn in agro-ecologische praktijken met *Vanilla* CWR's, natuurlijke bestuivers en agroforestry, en (b) bedrijven die op zoek zijn naar duurzaam geproduceerde en verhandelde vanillebonen, en geïnteresseerd zijn in marktdiversificatie. In deze context zullen boerencoöperaties waarschijnlijk een centrale rol spelen in capaciteitsopbouw, de organisatie van boeren op lokaal of regionaal niveau, en het verbinden met afzetmarkten.

Samengevat, deze thesis verschaft preliminaire inzichten inzake maatregelen die genomen kunnen worden om de geïdentificeerde problemen binnen de vanillesector te mitigeren, met name door het potentieel na te gaan van een strategie die het behoud en de duurzame teelt van *Vanilla* CWR's combineert. Door de verzamelde inzchten omtrent de verspreidingspatronen van *Vanilla* CWR's, hun natuurlijke bestuivers, en hun teeltpotentieel te combineren, konden we een schema ontwikkelen voor de praktische implementatie van de *Vanilla* SPASHA strategie in ACOSA – een strategie die overeenstemt met zowel het regionaal beleid rond landgebruik alsook de percepties van het merendeel van de ondervraagde boeren. Het is echter hoognodig om deze aanpak te evalueren in termen van productiehaalbaarheid, behoud van biodiversiteit, en marktintegratie van nieuwe vanillevariëteiten.

RESUMEN

El sector de la vainilla se enfrenta a varios retos que afectan negativamente su suministro estable. Procesos de domesticación han provocado una fuerte erosión genética del cultivo *Vanilla planifolia*. Además, las prácticas que implican monocultivos gestionados de forma intensiva, y las cadenas de suministro con una partición asimétrica de los ingresos, agravan aún más la vulnerabilidad de este sector. El Neotrópico alberga varias especies silvestres del cultivo de la vainilla (CWR). Al igual que la *V. planifola*, los *Vanilla* CWRs producen frutos, conocido como vainas, aromáticas. Además, los *Vanilla* CWR contienen una diversidad genética con potencial para su uso en la mejora de los cultivos, sin embargo, están en peligro de extinción. Existe una necesidad urgente de proteger estas especies de *Vanilla*, y de evaluar simultáneamente su posible contribución para contrarrestar los problemas de producción. El objetivo de la presente tesis doctoral es proporcionar información sobre el uso potencial de los *Vanilla* CWR dentro de un enfoque que pretende un esfuerzo conjunto de (a) la conservación del hábitat natural de los *Vanilla* CWR y sus dispersores de polen y semillas, y (b) el cultivo sostenible de vainilla en sistemas agroforestales alrededor de estas áreas protegidas, promoviendo la integración de los *Vanilla* CWR prometedores y sus polinizadores. Lo definimos como SPASHA (*joint land sparing/sharing*), según el cual la vainilla se comercializan idealmente a través de cadenas de suministro directas y en las que participan agricultores organizados y empresas responsables. Llevamos a cabo un estudio interdisciplinario para explorar la posible implementación de esta propuesta en nuestro región de estudio Área de Conservación Osa (ACOSA) – un punto crítico de biodiversidad en el suroeste de Costa Rica que alberga varias poblaciones de *Vanilla* CWR. La presente tesis desarrolló cuatro paquetes de trabajo para adquirir información sobre la distribución, los mecanismos de polinización, y el potencial de cultivo de los *Vanilla* CWR, así como sobre las percepciones de los miembros de la cadena de suministro hacia las innovaciones presentadas.

En el primer paquete de trabajo (Capítulo 2), se desarrollaron modelajes de distribución de las especies de *Vanilla* para proporcionar información sobre la posible implementación de SPASHA. Los mapas, modelados a la escala de Costa Rica, mostraron una variación inter-específica en relación con la idoneidad, muy probablemente influenciada por los rasgos funcionales específicos de la especie o por ciertas interacciones bióticas. ACOSA demostró ser adecuado para los cuatro *Vanilla* CWR modelados, y los mapas de idoneidad se superpusieron a los mapas de uso del suelo para definir (a) las áreas de conservación de baja y alta prioridad, es decir, las áreas boscosas con o sin estatus de protección, ambas con alta idoneidad para la vainilla, y (b) las

áreas potenciales de cultivo, es decir, las tierras degradadas que podrían reforestarse con la integración de *Vanilla* CWR prometedores, o las tierras agrícolas existentes que podrían transformarse en sistemas agroforestales con vainilla como cultivo comercial. En este contexto, las zonas de cultivo sirven de corredores biológicos entre las zonas de conservación, fomentando el intercambio de servicios ecosistémicos como la polinización entre el bosque y las tierras de cultivo. Las recomendaciones espaciales resultantes de este estudio encajan en el programa nacional de bio-corredores que busca crear prácticas sostenibles dentro de la red de corredores.

En el segundo paquete de trabajo (Capítulos 3 y 4), evaluamos la posible integración de los polinizadores naturales en los sistemas de cultivo de la vainilla. Identificamos los polinizadores y los mecanismos de polinización, examinamos los requisitos del hábitat de los polinizadores, y evaluamos la fructificación natural. Descubrimos que *Vanilla pompona* es polinizada por *Eulaema cingulata* y muestra un mecanismo dual de atracción de polinizadores que combina recompensas de fragancias florales con el engaño de alimento para inducir la extracción de polen. *Vanilla hartii* recompensa a sus visitantes florales con néctar alto en sacarosa y es polinizada por especies de *Euglossa* que encajan morfológicamente con las flores. Combinando nuestros resultados con una profunda revisión de la literatura, suponemos que las especies fragantes y neotropicales de *Vanilla* son polinizadas por Euglossini, y que las Euglossini han demostrado unos requerimientos de hábitat específicos. En general, la escasa fructificación natural, incluso para las especies con néctar, favorece la continuación de la polinización manual. Sin embargo, recomendamos encarecidamente el desarrollo de paisajes agroecológicos que consistan en sistemas de cultivo de vainilla respetuosos con los Euglossini que rodeen a los refugios de Euglossini (i.e. bosques intactos), por lo que las vainas de vainilla producidas en estos paisajes podrían ser certificadas y vendidas en mercados especializados.

En el tercer paquete de trabajo (Capítulo 5), obtuvimos los primeros datos sobre el potencial de cultivo de los cuatro *Vanilla* CWR modelados. Establecimos sitios de campo experimentales tanto en tierras boscosas como en agro-sistemas existentes (plantaciones de cacao) de ACOSA, y desarrollamos modelos que predicen la sobreviviencia, vitalidad y crecimiento de las plantas con las especies de *Vanilla* (los cuatro CWR, un híbrido de uso comercial y *V. planifolia*), los dos sistemas (bosque secundario y cacaotal), y varias variables ambientales. El híbrido y los CWR superaron a *V. planifolia* en términos de vitalidad, mientras que este último y el híbrido tuvieron la mayor tasa de crecimiento. El rendimiento de cada especie, sin embargo, dependió del sistema y de la intensidad de la luz, lo que nos permitió identificar las preferencias específicas de cada especie. Las bacterias supresoras de enfermedades y promotoras del crecimiento afectaron

positivamente a la supervivencia, la vitalidad y el crecimiento de las plantas. Además, observamos efectos significativos de ciertas variables químicas del suelo y rasgos funcionales del árbol tutor sobre el rendimiento de la planta de vainilla, lo que pone de relieve las importantes interacciones entre la vainilla y su entorno. Además, el potencial aromático cuantificado de las vainas de las diferentes especies demuestra las posibilidades de diversificación del mercado. Nuestros resultados muestran que los *Vanilla* CWR tienen potencial para ser incluidos en sistemas de cultivo que imitan su hábitat natural. Sin embargo, es necesario seguir investigando sobre la viabilidad (mano de obra, producción, rendimiento) y la disposición espacial de los cultivos.

En el último paquete de trabajo (Capítulo 6), llevamos a cabo un *discrete choice experiment* con 186 agricultores de ACOSA, para comprender mejor las perspectivas de los miembros de la cadena de suministro hacia las innovaciones en el sector de la vainilla. Combinando nuestros resultados con un estudio sobre las preferencias del mercado, vemos un gran potencial en el desarrollo de una cadena de suministro innovadora entre (a) los agricultores cooperativos y interesados en las prácticas agroecológicas que implican a los *Vanilla* CWR, los polinizadores naturales y la agroforestería, y (b) las empresas que buscan un abastecimiento sostenible de vainilla y quieren diversificar el mercado de vainilla. En este contexto, es probable que las cooperativas de agricultores desempeñen un papel fundamental en el desarrollo de capacitaciones, la organización de los agricultores, y la provisión de salidas comerciales.

En conclusión, proporcionamos una visión inicial de las posibles medidas para mitigar los problemas medioambientales y socioeconómicos identificados dentro del sector de la vainilla, al sondear el potencial de una estrategia que combina la conservación y el cultivo sostenible de los *Vanilla* CWR. Combinando nuestros datos en relación con los patrones de distribución de los *Vanilla* CWR, los polinizadores naturales y el potencial de cultivo en los agro-sistemas que pueden funcionar como corredores biológicos entre las áreas protegidas que albergan poblaciones silvestres de *Vanilla*, desarrollamos un esquema con potencial para la aplicación del enfoque SPASHA, específico para el caso del cultivo de vainilla en ACOSA. Es un enfoque que coincide con las políticas de uso de la tierra de este punto caliente de biodiversidad, así como con las percepciones de una gran parte de los agricultores cuestionados. Sin embargo, es muy necesaria una evaluación de este enfoque en términos de viabilidad de la producción, conservación de la biodiversidad e integración en el mercado de nuevas variedades de *Vanilla*.

LIST OF TABLES

Chapter 2

Table 2.1. Overview of land use recommendations based on the final map indicating unsuitable/suitable areas for vanilla cultivation overlaid with recent land use maps of the area and the current conservation status 39

Table 2.2. Overview of the mean AUC values for the four vanilla species and the AUC values of the individual algorithms with the highest and lowest performance.....40

Table 2.3. Overview of the contributions, with values ranging from 0 to 1, of the individual predictor variables included in the SDMs towards the distribution of the four vanilla species, and the total contribution of soil, precipitation, temperature and topography for each vanilla species 40

Chapter 3

Table 3.1. Percentage of landing bees per genus displaying a defined reward approach type on different parts of *Vanilla pompona* flowers (T = tepals; LE = labellum edge; LT = labellum tube), whereby “probing” refers to touching the flower surface with the proboscis and antennae and / or brushing with foretarsi, and “nectar search” refers to entering straight into the labellum chamber, and showing an extended tongue when entering or departing the flower 61

Table 3.2. Mean fruit set (%) of *Vanilla pompona* populations at the three study sites in Costa Rica, measured during the flowering period of 2019 and 2020. *SD* = standard deviation 62

Table 3.3. Identified floral volatile compounds, Kovats retention indices, retention times (min), and mean relative peak areas (%) calculated from the total peak area in the GC-MC chromatogram from *V. pompona* flowers (N = 12).....63

Chapter 4

Table 4.1. Overview of the proposed pollination strategies, pollinators, and reproductive success (fruit set (%)) of *Vanilla* species studied so far, grouped by subgenus and section according Soto Arenas & Cribb (2010) and Karremans et al. (2020). All pollinator species are bees belonging to the Apidae family, except for *V. saimensis*, which is pollinated by Halictidae 89

Chapter 5

Table 5.1. Model estimates of the logistic plant survival model 109

Table 5.2. Model estimates of the logistic mixed effect vitality_1 model 110

Table 5.3. Model estimates of the linear mixed effect vitality_2 model 111

Table 5.4. Model estimates of the linear mixed effect growth model 112

Table 5.5. Flower probability per *Vanilla* species after one and a half to two years of planting, demonstrating the average number of inflorescences and flowers produced per plant.....116

Table 5.6. Mean contents of main phenolic and anisyl compounds analysed by HPLC expressed in g·100 g^{-1} dry weight in mature vanilla pods of the different *Vanilla* species and hybrids.....118

Chapter 6

Table 6.1. Overview and description of selected attributes and corresponding levels 136

Table 6.2. Estimation results from the basic MXL model.....140

Table 6.3. Estimation results from the LC model with four latent classes 141

Table 6.4. Comparison of farmer characteristics across four mutually exclusive preference classes.....144

LIST OF FIGURES

Chapter 1

Figure 1.1. Gene pool concept of Harlan & de Wet (1971), adapted from Hammer et al. (2003) 10

Figure 1.2. The land sparing versus land sharing debate based on the biodiversity/production response, and adapted from Salles et al. (2017), whereby (a) a concave response favours land sharing and a convex response promotes land sparing (Green et al., 2005; Phalan et al., 2011), or (b) a mixed strategy of land sharing and sparing is recommended (Teillard et al., 2015) 13

Figure 1.3. Visualisation of the combined land sparing and land sharing (SPASHA) approach proposed for the vanilla crop, whereby sustainable vanilla cultivation systems (e.g. agroforestry systems) may allow the exchange of essential ecological interactions between *Vanilla* plants and their environment, while they simultaneously provide a connection between the surrounding natural habitats necessary to protect the wild populations of *Vanilla* CWRs 15

Figure 1.4. The general model of the vanilla supply chain (Yoon et al., 2020) 18

Figure 1.5. Historical boom and bust price cycles typical for the vanilla industry. Since the liberation of the vanilla market in the 1990s, vanilla prices have been highly volatile. In 2000, hurricanes damaged the majority of Madagascar's plantations, leading to an international price rise up to about USD 500 / kg in 2003. From 2004 onwards, there was an overabundance resulting in price drops. Due to the low revenues, vanilla farmers abandoned their plantations, leading to vanilla shortages in 2012-2013 and thus an increase in prices. Typhones in 2017 and 2018, however, caused a further rise in prices with records up to USD 500 / kg and more. Prices are expected to remain high for the coming years, as vanilla farmers are still recovering from the damages caused by these extreme weather events (Kunz et al., 2020)……..…….19

Figure 1.6. Schematic overview of the four work packages, each with its own specific objective, embedded within the proposed *Vanilla* SPASHA approach and corresponding supply chain 21

Figure 1.7. Map of our study area, Área de Conservación Osa (ACOSA), in southwest Costa Rica. Corcovado and Piedras Blancas are the two terrestrial national parks, and the Golfo Dulce reserve connects these two national parks. Golfito wildlife refuge borders Piedras Blancas national park, and the biological corridor Osa is part of the National Bio-Corridor Program (PNCB) of Costa Rica 24

Chapter 2

Figure 2.1. The concept of joint land sparing/sharing (SPASHA) explained for vanilla conservation and cultivation, allowing for natural interactions such as pollination between the forest and neighboring vanilla agroforestry systems 33

Figure 2.2. Map displaying the corridors that are expected to be suitable for *V. odorata* focusing on our study region ACOSA and the corridor Osa. The light green areas are the areas suitable for the species in question to occur 42

Figure 2.3. Map highlighting areas for conservation and sustainable cultivation of *V. odorata* within the ACOSA region. The green areas are forests suitable for vanilla to occur, giving these areas a vanilla conservation status, but the priority further depends on the location (in- or outside protected areas such as national parks). The brown and yellow areas are respectively plantations and grazing lands recommended for the implementation of sustainable agroforestry systems (AFS) with vanilla as a cash crop, especially in areas where natural vanilla populations (potentially can) occur in the nearby forests. The white areas are unsuitable areas for vanilla to occur, so with no recommendations regarding vanilla conservation or cultivation 43

Chapter 3

Figure 3.1. Map showing the study sites in (a) Osa Peninsula, Costa Rica (Miramar, Piro), and (b) Madre de Dios region, Peru (Baltimore, Kapievi, Belo Horizonte). Both areas include various protected areas such as national parks, communal or national forest reserves, and biological corridors.....56

Figure 3.2. Measured *V. pompona* floral traits: height (H1), width (W) and length (L1) of the labellar tube, height of the labellar tube below the anther (H2), thickness of the column at the height of the anther (T_Co), height of the labellar callus (T_Ca), height of the labellar keels (T_La), and length of the pedicel (L2). (vouchers at JBL) 60

Figure 3.3. Caught male *Eulaema cingulata* with attached *V. pompona* pollen and extended tongue (A), and attached pollen of *V. pompona* in close-up. (vouchers at JBL).....62

Figure 3.4. Comparison of the mesosoma heights (mm) of the bee species *Eulaema bombiformis*, *Eulaema meriana*, *Eulaema cingulata* and *Euglossa* spp. with the flower morphological traits “An” (mm) and “Cn” (mm) of *V. pompona* 64

Chapter 4

Figure 4.1. The Osa Peninsula, our study area in southwest Costa Rica, with indications of the four study sites with a *Vanilla hartii* population: (1) Bambu, (2) Banegas, (3) Los Mogos, (4) Piro 77

Figure 4.2. *Vanilla hartii* flower (A), and *V. hartii* flower with removed sepals and petals to indicate the nectary (white arrow) (B) and the measured floral traits: L1 = length of labellar tube, L2 = length of pedicel, T_co = thickness of column at anther height, T_la = height of labellar keels, T_ca = height of labellar callus, H = height of labellar tube below anther, W = width of labellar tube (C). (vouchers at JBL) 78

Figure 4.3. Comparison of (A) the nectar volumes between the four *V. hartii* populations, (B) the contribution of each of the identified nectar sugars present in *V. hartii* nectar and (C) the *V. hartii* nectar sugar composition among the four populations using non-metric multidimensional scaling, with Fru = fructose, Glu = glucose, Ra = raffinose, Su = sucrose 82

Figure 4.4. Comparison of the mesosoma heights (mm) of the caught *Euglossa* spp. with the flower morphological traits “An” (mm) and “Cn” (mm) of *Vanilla hartii*. The potential pollinator species, i.e. the *Euglossa* species with a mesosoma width smaller than the diameter of the labellar tube and a height that fits into the “An-Cn” gap, are shown in green 83

Chapter 5

Figure 5.1. The location of the twelve experimental sites, randomly spread across the study area ACOSA. The orange and red dots represent the sites in secondary forests and cacao plantations, respectively 101

Figure 5.2. Planting design in traditional cacao plantations (3 m x 3 m) (A), and naturally regenerated secondary forests (B). The numbers (1-6) refer to the six rows per site, in which each row contains one individual of each *Vanilla* species, following a random planting design across the site (C) 101

Figure 5.3. Predicted effect of land use system on vanilla plant survival (A), vitality of the planted cutting (B), vitality of the new shoots (C), and vanilla plant growth (D), given per Vanilla species and coloured according group (CWR, crop species, hybrid) 113

Figure 5.4. Predicted interaction effect of incoming light (%) on vanilla plant survival (A), vitality of the planted cutting (B), vitality of the new shoots (C), and vanilla plant growth (D), given per *Vanilla* species 113

Figure 5.5. Predicted effect of soil bacterial diversity (Shannon index) on vanilla plant survival (A), vitality of the planted cutting (B), and vanilla plant growth (C) 114

Figure 5.6. Predicted effect of the relative abundance of specific bacterial phyla vanilla plant survival (A, B), vitality of the planted cutting (C), and vanilla plant growth (D, E, F) 114

Figure 5.7. Predicted effect of the vitality of the planted cutting and soil pca1 on the vitliaty of new shoots (respectively A and B), and soil pca2, SLA of the tutor trees, and leaf C:N ratio on vanilla plant growth (respectively C, D and E) 115

Figure 5.8. PCA plot displaying the aromatic compound composition among the studied *Vanilla* species, based on the eight volatile compounds (vanillin, vanillic acid, vanillyl alcohol, pHB, pHB acid, pHB alcohol, anisyl acid, anisyl alcohol) determined by HPLC 117

Chapter 6

Figure 6.1. Example of a choice card used during the DCE, showing the two alternatives (option A and B), differentiated by the levels of the six attributes, and an opt-out option (status-quo) .138

Figure 6.2. Map showing the distribution of the four farmer classes within the surveyed area 143

Figure 6.3. Schematic overview of the proposed two-tier approach for the implementation of a vanilla cultivation program in a high-priority conservation area such as the Península de Osa, taking into account the distinct differences in preferences among the farmers with interest in vanilla cultivation 149

Chapter 7

Figure 7.1. Schematic representation of (A) the dual pollinator attraction mechanism in *V. pompona* and (B) the nectar-rewarding pollination mechanism in *V. hartii* 161

Figure 7.2. Schematic representation of the formulated hypothesis linking the observed *Vanilla* species distribution patterns with pollinator requirements 164

Figure 7.3. Possible design for the *Vanilla* SPASHA approach that (a) promotes the presence of forest patches (*Vanilla* and Euglossini refuges) surrounding Euglossini-friendly vanilla cultivation areas to enhance possible pollinator exchange between forest and farmland, and (b) takes into account the observed *Vanilla* species-specific preferences that allow optimal vanilla plant performance within the cultivation areas. The implementation could be carried out on a fallow or degraded land (left) or on existing agricultural lands such as a cacao plantation (right). The latter will require a spatial transformation of current designs (3 x 3 m) 170

Figure 7.4. Schematic overview of potential implementation of the *Vanilla* SPASHA approach within our study region ACOSA, based on the results of the SDM (Chapter 2), pollination studies (Chapters 3-4), and the field experiment (Chapter 5) 171

Figure 7.5. Potential supply chain schemes for the proposed mainstream vanilla production approach and the *Vanilla* SPASHA approach 174

Figure 7.6. Schematic overview of the main conclusions.....176

LIST OF ABBREVIATIONS

ACOSA	Área de conservación Osa
AFS	Agroforestry system
AIC	Akaike's information criterion
ANOSIM	Analysis of similarities
ASC	Alternative specific constant
ASV	Amplicon sequence variant
AUC	Area under receiver operating characteristic curve
B2B	Business to business
B2C	Business to consumer
BIC	Bayesian information criterion
BIEN	Botanical information and ecology network
BRT	Boosted regression trees
C	Carbon
Ca	Calcium
CAIC	Consistent Akaike information criterion
CP	Cacao plantation
CRC	Costa Rica colon
Cu	Copper
CWM	Community weighted means
CWR	Crop wild relatives
DBH	Diameter at breast height
DCE	Discrete choice experiment
FAMD	Factor analysis of mixed data
FDA	Flexible discriminant analysis
Fe	Iron
FONAFIFA	Fondo nacional de financiamiento forestal
FSC	food supply chain
GAM	Generalized additive models
GBIF	Global biodiversity information facility
GC-MS	Gas chromatography-mass spectrometry
GHG	Greenhouse gas
GLM	Generalized linear models
GMTED2010	Global multi-resolution terrain elevation data 2010

Gp	Gene pool
HPLC	High-performance liquid chromatography
IBPGR	International board for plant genetic resources
ISRIC	International soil reference and information centre
ITPGRFA	International Treaty on Plant Genetic Resources for Food and Agriculture
JBL	Jardin Botanico Lankester
K	Potassium
LC	Latent class
LDMC	Leaf dry matter content
LOD	Limits of detection
LOO	Leave-one-out cross-validation
MCA	Multiple correspondence analysis
MDG	Millennium development goal
Mg	Magnesium
MGCV	Multivariate adaptive regression splines
MINAE	Ministerio de ambiente y energía
Mn	Manganese
MXL	Mixed logit
N	Nitrogen
NMDS	Non-metric multidimensional scaling
P	Phosphor
PAR	Photosynthetic active radiation
PCA	Principal components analysis
PES	Payments for ecosystem services
PFPS	Perfume Flower Pollination Syndrome
pH	Potential of hydrogen
pHB	p-hydroxybenzaldehyde
PNCB	Programa nacional de corredores biológicos
REML	Restricted maximum likelihood
RF	Random forests
rRNA	Ribosomal RNA
S/N	Signal-to-noise ratio
SD	Standard deviation
SDG	Sustainable development goal

SDM	Species distribution modelling
SE	Standard error
SF	Secondary forest
SFSC	Short food supply chain
SINAC	Sistema nacional de áreas de conservación
SLA	Specific leaf area
SMTA	Standard material transfer agreement
SPASHA	Joint land sparing / sharing
SSCM	sustainable supply chain management
SVM	Support vector machines
USD	United States dollar
VIF	Variation inflation factor
VOC	Volatile compound
WAIC	Widely applicable information criterion
Zn	Zinc


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Chapter 1

Introduction

Photo: Vanilla beans of the hybrid (*Vanilla planifolia* x *Vanilla pompona*) x *Vanilla planifolia*, embraced by a farmer living in our study region in southwest Costa Rica. © Charlotte Watteyn

1.1 CONTEXT AND PROBLEM STATEMENT

1.1.1 Food production and its environmental and social impact

If there is one species that has been transforming the Earth's surface, atmosphere and oceans in an extensive way, it is our own *Homo sapiens*. Human-induced environmental impacts started as long as 50,000 years ago (Boivin et al., 2016; Ellis et al., 2021; Cepic et al., 2022). Yet, modern rates and scales are far greater than those of the past (Klein et al., 2011; Steffen et al., 2015; Watson et al., 2018; Stephens et al., 2019). The endeavour to satisfy our food demands and hunger for economic growth provoked large-scale destruction and degradation of natural habitats, with the latter being largely transformed to massive agricultural and industrial areas (Davies et al., 2001; Tilman et al., 2001; Asafu-Adjaye, 2003; Laurance, 2010). Our continuous pressure on the natural environment has led to the disruption of long-standing equilibria, ensuing the contemporary observed problems related to climate change, biodiversity loss, soil degradation, and reduction of water and air quality, amongst others (Gavinet, 2020). The human population is expected to reach at least 9 billion by the year 2050, requiring up to 70% more food, and imposing even higher challenges to terrestrial and aquatic systems (Foley et al., 2011; King et al., 2017; Roberts & Mattoo, 2019).

Present-day global food production and trade systems, defined as the production, processing and distribution of food across the world (Gladek et al., 2017; von Braun et al., 2021), are often far from sustainable, particularly those associated with large-scale industrial farming (Nicholls et al., 2020). Although the agricultural sector is very vulnerable to climate change, and has the potential to be an important actor towards climate change adaptation and mitigation, it is currently one of its main drivers, accounting for about one-fifth of the total greenhouse gas (GHG) emissions (Springmann et al., 2018). Deforestation for agricultural purposes accounts for an extra 10-11% of global GHG emissions (Struik et al., 2017). Moreover, modern farming systems generally consist of crop and livestock species with a narrow genetic base (Khoury et al., 2014), mostly produced in monoculture systems, and thereby heavily undermining the biodiversity and associated ecosystem services they actually depend upon (Nicholls et al., 2020). This ecological homogeneity may maximize productivity and efficiency, but simultaneously reduces the resilience to environmental and socio-economic alterations (Tilman et al., 2001; Foley et al., 2011; Zampieri et al., 2020). The path along which agricultural products are then globally traded towards its end-consumers also raises certain concerns. Notwithstanding the rising demand for a better integration of social equity in global food supply chains, the latter are still known to be an asymmetric process, whereby capital accumulates to some actors in some places that are

generally not the primary production or processing countries (Werner, 2019; Bair et al., 2021; Basu & Vasudevan, 2021). Building more resilience and sustainability in both production and trade systems will be key to secure the long-term viability of the food sector.

The 2030 Agenda for Sustainable Development announced 17 universal goals and 169 targets referred to as the Sustainable Development Goals (SDGs), which are built upon the Millennium Development Goals (MDGs) (Sachs, 2012; UN SDG, 2018) and encourage action in areas focusing on sustainable development by poverty eradication. The MDGs comprised a set of eight goals that were targeted towards traditionally marginalized groups such as ethnic minorities, indigenous groups, and women. The SDGs include a more global approach, with an agenda spanning from 2015 to 2030. They strive to decouple economic growth from environmental degradation, to ensure an efficient management of natural resources, and to establish a fair distribution of benefits across society (Sayer & Cassman, 2013). Within this context, sustainable agriculture has great potential to achieve multiple SDGs, such as zero hunger, responsible consumption and production, climate action, and life on land. The current separation between biodiversity conservation and agricultural production has, however, been an impediment to achieving adequate outcomes (Gordon et al., 2017). As such, a true societal transformation will be crucial to guarantee both food security and equal capital distribution, while preserving the remaining integrity of the biosphere and minimizing the negative impacts on ecosystem services and biodiversity (Roberts & Mattoo, 2019; Folke et al., 2021).

1.1.2 Let's spice it up: The vanilla sector as an example

Vanilla is a precious tropical food commodity, and although used in rather small quantities, it is present in a wide range of food, cosmetic, and pharmaceutical products. The spice is derived from the fruits, better known as pods or beans, of orchids belonging to the pantropical genus *Vanilla* Plum. ex Mill., a monophyletic primitive lineage of the Orchidaceae family (Cameron, 2011). *Vanilla* orchids naturally occur in different habitat types across tropical America, Asia and Africa (Portères, 1954). They have aerial roots with a function in both water and mineral absorption as well as in the attachment to support trees (Cameron, 2011). The earliest documented cultivation of vanilla beans dates back to the Totonac empire in Mexico, where naturally pollinated beans were traded and used for perfume, cacao flavouring, medicine, and spiritual gatherings (Havkin-Frenkel & Belanger, 2018). Vanilla was integrated into a swidden agriculture wherein *milpa* (maize) fallows were managed as agroforests involving vanilla support trees (e.g. fruit or timber trees) and other crop species (Toledo et al., 2003). Soon after the

Spanish conquest in 1519, some vegetative material was taken to Europe, where cultivation attempts failed due to unfavourable climatic conditions. Vanilla cuttings were therefore brought over to former European colonies in the Indian Ocean (Lubinsky et al., 2008, Hernández & Lubinsky, 2010). It was here that plants started to flower, though no fruit set was observed due to the absence of natural pollinators. The discovery of a manual pollination technique in 1841, however, instigated a vanilla cultivation boom in this geographical area (Havkin-Frenkel & Belanger, 2018), with Madagascar and Indonesia being the leading production countries to date (Faostat, 2021). Manual pollination not only enables commercial production in absence of natural pollinators, but also allows farmers to obtain satisfying yields by manipulating the amount of flowers being pollinated. This labour-intensive technique is now widely applied in commercial vanilla plantations. Despite its benefits, manual pollination often results in over-pollination, resulting in smaller or deformed fruits of lower value. Also, the high energy levels being invested in fruit development may lead to plants with a lower resilience against biotic and abiotic stresses (Cameron, 2011; Havkin-Frenkel & Belanger, 2018).

The fact that vanilla is propagated in a vegetative way, has caused a genetic bottleneck within the widely used crop species *Vanilla planifolia* Jacks. ex Andrews, characterized by increased susceptibilities towards pathogen infestation and lower resilience to environmental changes. Furthermore, since pollen is placed on the stigma of the same flower, outcrossing rates are negligible, confining the genetic variability within the crop population (Flanagan & Mosquera-Espinosa, 2016). This limited cop genetic base as well as the widespread implementation of monocultures exacerbate the low resilience of vanilla cultivation systems even more (Bory et al., 2008; Flanagan & Mosquera-Espinosa, 2016). Besides production problems, also the vanilla supply chain faces certain challenges. Since mainly two countries are monopolizing the production, there is not only a lack of resilience against extreme climate events or proliferating diseases, but also against political instability, theft, and processing errors (e.g. early green bean picking and quick-curing). Moreover, the supply chain is complex and involves numerous stakeholder groups, starting with vanilla farmers and collectors at community or regional level, followed by various curers, exporters, international brokers, and finally wholesalers or extract manufactures (Ecott, 2004; Yoon et al., 2020). The historical path of unsustainable practices, price volatility, low farm-gate prices, and collusion among traders has labelled the vanilla sector as being highly unstable (Munshi, 2020).

Considering the production and socio-economic problems, innovations within the vanilla sector are highly recommended. First, a better understanding of the **genetic pool** available among the

different *Vanilla* species, and their potential use within the agricultural sector, should be given priority - as they provide the diversity necessary to cope with current challenges related to the crop's genetic erosion (Heywood et al., 2007; Dempewolf et al., 2014, 2017; Chambers et al., 2021; Goettsch et al., 2021). Second, the development of **resilient vanilla cultivation systems** is vital to diminish the sector's vulnerability to climate change, price volatilities, and product insecurity. If implemented at the landscape scale, these cultivation systems can contribute to the establishment of robust, multifunctional land use strategies that strive poverty alleviation, enable sustainable development and support climate change adaptation and mitigation efforts (Sayer et al., 2013). Third, the **empowerment of vanilla farmer communities** and the strengthening of global vanilla supply chains, by building solid alliances among all actors involved, guarantees a more equal revenue partition along the chain (Vroegindewey & Hodbod, 2018; Wang et al., 2021).

The abovementioned aspects are discussed in the following sections. In essence, this PhD thesis seeks to explore the potential contribution of the wild relatives of the vanilla crop, and this within a land use strategy that combines their conservation and sustainable cultivation, and eventually leads to the development of a resilient vanilla supply chain. Prior to the description of the research aim and objectives, we describe (a) the current state of knowledge regarding *Vanilla* crop wild relatives, (b) the concept of joint land sparing/sharing as a possible strategy for sustainable vanilla production, orchestrating human land use with biodiversity conservation, and (c) the problematic situation of the vanilla supply chain, highlighting possible improvements that could promote a more sustainable supply of high-quality vanilla beans.

1.2 BACK TO THE WILD WITH CROP WILD RELATIVES

1.2.1 Current state of knowledge on crop wild relatives

For centuries, farmers have been improving crop quality and yield through traditional breeding methods – the detailed selection of valuable agronomic traits known as crop domestication (Gepts, 2004; Meyer et al., 2012). Yet, it was not until more recently, e.g. via initiatives such as the Green Revolution, that the dominating germplasm shifted from landraces or locally adapted varieties that farmers had been selecting over time, to modern varieties and more widely adapted seed types (Hammer, 1984; Tanksley & McCouch, 1997; Gross & Olsen, 2010; Meyer & Purugganan, 2013; Pingali, 2019). The latter doubtlessly satisfies the high demand for productivity and uniformity but generally narrows crop genetic diversity, as it filters valuable genetic variants and associated phenotypes out of the crop's gene pool (Dempewolf et al., 2017; Martin et al.,

2019; Khoury et al., 2021). This in turn may lead to food production systems with low resilience to environmental changes, a trend that has been observed in a range of crop species (Ross-Ibarra et al., 2007; van Heerwaarden et al., 2011).

It is about time to reappraise the value of crop diversity, with a special focus on the great genetic pool available among wild plant species. This recognition dates back to the beginning of the 20th century (e.g. work of Vavilov, Harlan, de Wet, Bennett, Frankel, Hawkes) (Pistorius, 1997; Meilleur & Hodgkin, 2004; Heywood et al., 2007), but only a few decades ago, and mainly as a consequence of increasing production problems related to climate change, considerably more attention has been paid to these so-called crop wild relatives (CWRs). By definition, CWRs are wild plant species that are genetically related to domesticated crops (Maxted et al., 2006). Following the gene pool concept of Harlan & de Wet (1971) (Fig. 1.1), this kinship can occur at three levels. The primary gene pool consists of the cultivated plants themselves and their wild forms, i.e. the same species but with a distinct evolutionary track leading to substantial genetic differences between these individuals. The second gene pool are species that are less associated with cultivated plants, i.e. wild relatives that belong to the same genus or clade but are more difficult to reproduce using conventional breeding techniques, although gene transfer to cultivated plants is possible. The third gene pool also consists of species that are associated with cultivated plants but, contrastingly to the second gene pool, advanced engineering techniques are needed to perform gene transfer. CWRs have passed through a long evolutionary history across various environments, thereby enduring several types of stresses. They are expected to be better adapted to extreme climates, adverse soil types, pests and diseases (Guarino & Lobell 2011; Khoury et al., 2020). Hence, CWRs are fundamental for global food security as they are the main source of novel genetic diversity necessary for crop improvement, including resistance genes to biotic and abiotic stress, novel flavours and nutrients, and improvements in plant architecture and yield (Tanksley & McCouch, 1997; Jarvis et al., 2008; Brummer et al., 2011; Dempewolf et al., 2017).

CWRs have been used successfully in past plant breeding, and contributed to the development of beneficial agronomic and nutritional crop traits (Hajjar & Hodgkin 2007). This includes major and minor cereals, root and tuber crops, oil crops, vegetables, fruits, forages and spices (Castañeda-Álvarez et al., 2016; Dempewolf et al., 2017). Despite the many efforts and success stories, knowledge gaps concerning CWR's taxonomy, relatedness to pertinent crops, geographic distribution, ecological interactions, and agriculturally relevant traits, currently constrain a thorough exploration of their potential use for crop improvement (Fowler & Hodgkin, 2004; Khoury et al., 2013; Dempewolf et al., 2017; Goettsch et al., 2021). These gaps also restrict the successful

development of both *in situ* and *ex situ* CWR conservation efforts, which are essential to (a) ensure their continued evolution, by protecting their wild populations as well as their ecological interactions (e.g. pollinators, seed dispersers) from land use and climate change, air and water pollution, invasive or other problematic species, or overexploitation in their natural habitats (Maxted et al., 2013; Ford-Lloyed et al., 2011; Hunter et al., 2012), and (b) guarantee a long-term protection and storage of these genetic resources, by making them available for research and education in gene banks and botanical gardens (Dempewolf et al., 2017; Benlioglu & Adak, 2019).

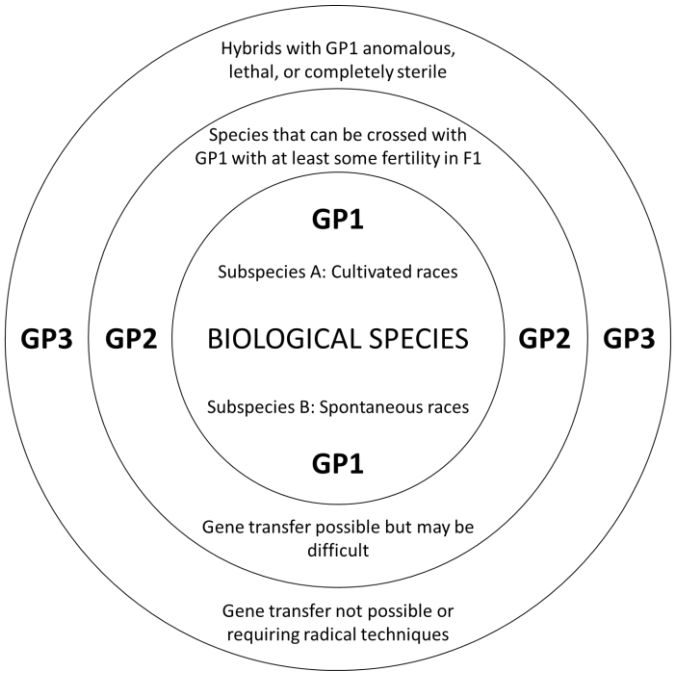

Fig. 1.1. *The gene pool concept of Harlan & de Wet (1971), adapted from Hammer et al. (2003).*

To date, approximately 50,000 to 60,000 plant species have been recognized as CWRs, of which 4,157 have potential or confirmed uses in crop improvement and food security (Dempewolf et al., 2017). Unfortunately, about 75% of these wild species are currently under treat due to human-induced environmental changes (Jarvis et al. 2008; Goettsch et al., 2021). To provide effective conservation strategies, it is necessary to recognize their centres of origin, i.e. locations where crops and their wild relatives are geographically co-located (Hummer & Hancock, 2015; Goettsch et al., 2021). From here, species can be characterized and, together with advances in breeding technologies, a more efficient CWR use can be achieved (Castañeda-Alvarez et al., 2016; Khoury et al., 2020). It is a long process that requires a global effort, involving several steps from *in situ* CWR localization, identification and collection, to *ex situ* conservation in gene banks, evaluation and validation of potentially interesting crop traits, and the establishment of breeding programs

for the development and trial of promising crop varieties (Castañeda-Alvarez et al., 2016; Dempewolf et al., 2017; Bramel & Frey, 2021).

1.2.2 *Vanilla* crop wild relatives

The genus *Vanilla* is considered to be around 65 million years old (Ramírez et al., 2007), supporting the hypothesis that its pantropical distribution resulted from the separation of continental landmasses. It is the most diverse genus within the subfamily Vanilloideae, and is divided into two subgenera: *Vanilla* and *Xanata*, with the latter being subdivided into two sections, *Xanata* and *Tethya*. Most are succulent hemi-epiphytic climbing vines, but they differ in habitat preference, foliage, floral structure, and ecological interactions (e.g. pollinators, seed dispersers, mycorrhizal symbiosis) (Cameron & Soto Arenas, 2003). Despite the presence of systematic controversy and confusion, recent studies have greatly enhanced our understanding of their classification, evolution, and origins. Soto Arenas & Cribb (2010) and Soto Arenas & Dressler (2010) recognized 110 *Vanilla* species, of which 51 are endemic to the Neotropics (subg. *Vanilla* and subg. *Xanata* sect. *Xanata*) and 59 are pantropical (subg. *Xanata* sect. *Tethya*). Since then, 20 additional species have been described for the Neotropics. The recent revision of Karremans et al. (2020), however, recognized a significant level of taxonomic inflation and reduced the number of Neotropical species back to 62, of which 38 belong to the sect. *Xanata*. The latter consists of the fragrant *Vanilla* species, i.e. species endemic to tropical America and producing fragrant vanilla beans that are of interest for the industry.

Notwithstanding the high diversity of fragrant *Vanilla* species, only a few are used within the agricultural sector, with *V. planifolia* being the dominantly cultivated species worldwide. As mentioned earlier, the vanilla crop is facing various production challenges, mainly due to genetic erosion and vulnerable cultivation systems. This in turn negatively affects the stable provision of high-quality vanilla beans. As highlighted by Flanagan and Mosquera-Espinosa (2016) and Bramel and Frey (2021), research on *Vanilla* CWRs is vital to combat present agronomic challenges as well as to access alternative market outlets. The wild relatives of the vanilla crop are growing in the moist lowland rainforests of the Neotropics, and have passed through a long evolutionary history across diverse environments (Flanagan & Mosquera-Espinoza, 2016; Goettsch et al., 2021). They are surviving in remaining habitat fragments and represent valuable sources of useful traits to upgrade vanilla cultivation in terms of climate change adaptation, disease resistance, pollination effectiveness, and aromatic quality. To date, most research has focused on the first gene pool of *Vanilla* CWRs, i.e. wild populations of the crop species *V.*

planifolia. This resulted in the identification of individuals with increased resistance to *Fusarium* (e.g. “Handa” variety) (Koyyappurath et al. 2015; Grisoni et al., 2021). Exploration within the secondary gene pool (i.e. fragrant *Vanilla* CWRs) resulted in a hybrid between *V. planifolia* and *V. pompona*, demonstrating increased resistance to *Fusarium*, and between *V. planifolia* and *V. odorata*, known for its distinct aromatic profile (Brunschwig et al., 2017; Grisoni et al., 2021). Many more traits are likely to be explored within the available gene pools, as demonstrated by Hasing et al. (2020) and Chambers et al. (2021) in their recent genomic-based diversity analysis that exposes the high genetic diversity among *Vanilla* accessions. Additionally, natural cross-pollination by members of the bee tribe Euglossini has been reported for some secondary gene pool *Vanilla* CWRs (e.g. Ackerman, 1983; Lubinsky et al., 2006; Householder et al., 2010; Soto Arenas & Dressler, 2010; Van Dam et al., 2010; Gigant et al., 2016; Pansarin & Pansarin, 2014; Anjos et al., 2017). Outcrossing augments intraspecific genetic diversity as it generates gene flow within and among populations (Gamba & Muchhala 2020; Ollerton 2021). In a nutshell, wild *Vanilla* populations are holding a diverse gene pool with traits that could be used for crop improvement and subsequent breeding programs.

1.3 TOWARDS A MULTIFUNCTIONAL LAND USE STRATEGY FOR THE VANILLA CROP

1.3.1 Land sparing versus land sharing

Debates on how to harmonize human land use with biodiversity conservation started more than 20 years ago (Pimental et al., 1992; Kendall & Pimental, 1994; Waggoner, 1996; Goklany, 1998). During the last years, research has paid substantially more attention to the development of integrated approaches that take into account the interlinkages between food security, conservation, and land restoration (e.g. Sayer et al., 2017; Reed et al., 2017, 2021; Kusters et al., 2020). Such approaches provide tools for the establishment of so-called multifunctional land use strategies that contribute to poverty alleviation, support climate change adaptation and mitigation efforts, and enable sustainable development (Sayer et al., 2013). Two examples of such land use strategies are land sparing and land sharing. Land sparing sets aside some land for conservation, while other land is used intensively for food production (Waggoner, 1996). The idea behind this strategy is that, by producing more food per unit of cultivated area, large tracts of land can be protected for nature’s exclusive use. Land sharing is an alternative strategy whereby the same land is assigned to conservation and crop cultivation, comprising a temporal and spatial spread of the provision of associated food commodities. Compared to land sparing, production techniques in a land sharing system are less intensive and allow a combination of food production

and biodiversity conservation. Additionally, several ecosystem services, such as carbon sequestration and enhanced soil productive capacity, are facilitated within these systems (Fischer et al., 2014; Phalan et al., 2011; Tscharntke et al., 2012; Wright et al., 2012). An example of a land sharing strategy is agroforestry – the interaction of woody perennials (trees, shrubs, palms, etc.) with crops and/or livestock on the same piece of land (Nair et al., 2021).

A strong controversy exists on which of these two strategies to follow, acknowledged as the "land sparing versus land sharing debate" (Green et al., 2005). In general, a land sparing or land sharing approach is preferred when the biodiversity/production response follows, respectively, a convex negative or a concave curve (Fig. 1.2). Biodiversity is hereby measured as species density, while production is measured as a combination of both yield and input intensity (Tilman et al., 2001; Kleijn et al., 2009; Phalan et al., 2014; Teillard et al., 2015).

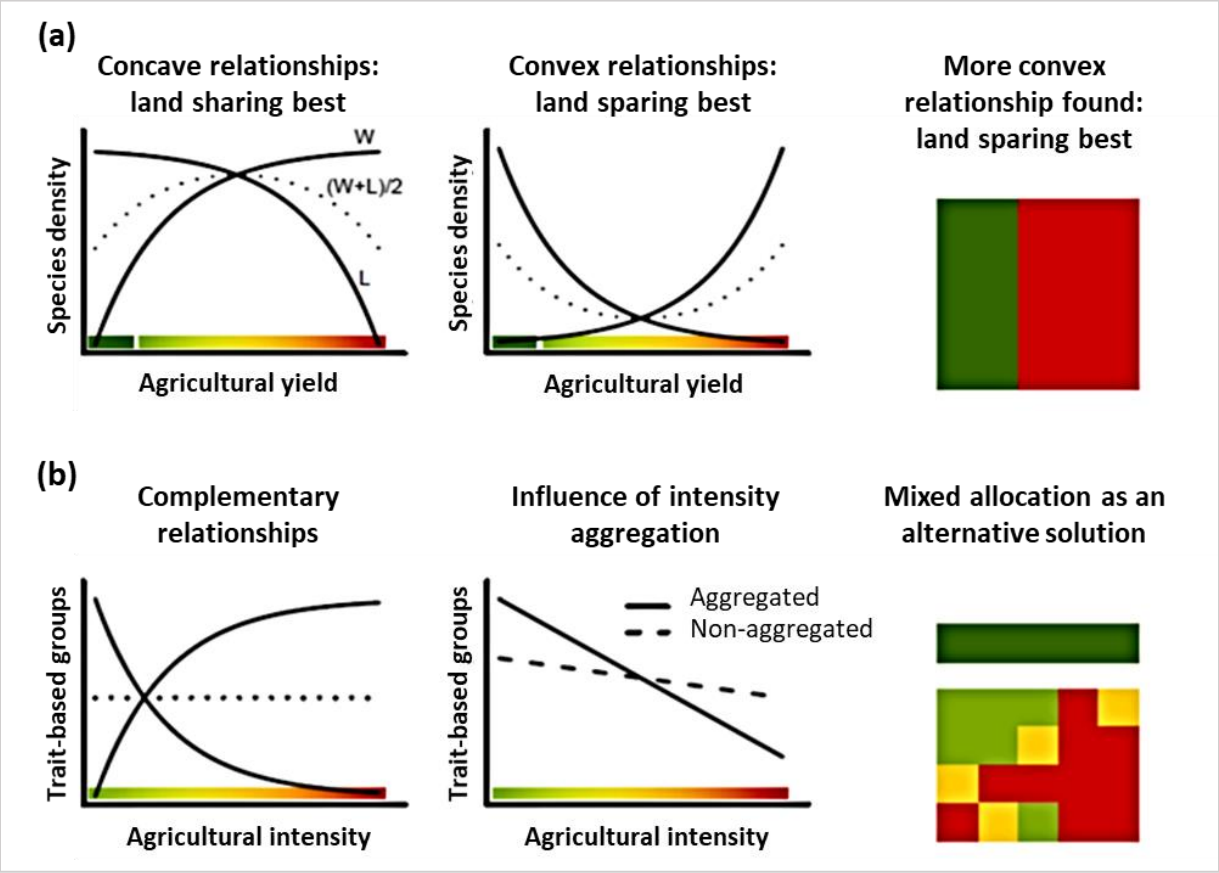

Fig. 1.2. *The land sparing versus land sharing debate based on the biodiversity/production response, and adapted from Salles et al. (2017), whereby (a) a concave response favours land sharing and a convex response promotes land sparing (Green et al., 2005; Phalan et al., 2011), or (b) a mixed strategy of land sharing and sparing is recommended (Teillard et al., 2015).*

Land sparing and land sharing are, however, not mutually exclusive and both approaches seem to be necessary to meet the rising food demand at the least cost to biodiversity (Fischer et al., 2008; Teillard et al., 2012; Scariot, 2013; Grass et al., 2019). Species move throughout the landscape and exploit different habitats, thereby increasing intraspecific diversity and thus resilience against changing environments, as individuals from one population meet with individuals from other populations. In a land sparing context, the necessary connectivity between these natural habitats may not be present due to the amid allocation of intensively managed farming areas, leading to habitat fragmentation and species movement obstruction. Contrastingly, in a land sharing context, the lack of fully protected natural areas may lead to the disappearance of species that depend on intact habitats during certain stages of their life cycle. For example, the spill-over of ecosystem service providers (e.g. pollinators, seed dispersers) coming from their so-called refuges to agricultural areas is of great benefit for food production (Tscharntke et al., 2005; Blitzer et al., 2012; Mitchell et al., 2013; Kremen, 2015; Kremen et al., 2018), but this may only occur in the presence of both protected lands as well as wildlife-friendly agricultural systems. Hence, a successful management of multifunctional landscapes may require the combination of context-specific land sparing and land sharing measures, providing a heterogenous and spatially interconnected landscape of sustainably managed and protected areas (Grass et al., 2019).

1.3.2 A combined land sparing/sharing approach for *Vanilla* conservation and cultivation

Combining the abovementioned land sharing and land sparing concepts in a so-called joint land sparing/sharing (SPASHA) approach may be favourable for certain crop species, especially if cultivated in areas of high conservation value (Crespin & García-Villalta, 2014). In this context, wildlife-friendly managed agroforestry systems can function as productive interlinkages between natural areas, thereby merging conservation with socio-economic development. An example of a crop for which SPASHA could be an interesting approach is vanilla, an understorey forest climber that can easily be introduced into agroforestry systems resembling their natural habitat. In particular, SPASHA could be of added value in the center of vanilla crop origin, as it may (a) assure the *in situ* conservation of its wild relatives, by protecting the natural habitats in which these *Vanilla* CWRs occur, and thereby safeguarding the gene pool of this high-value spice, and (b) enable the sustainable use of the *Vanilla* gene pool, by including compelling CWRs into vanilla cultivation systems that mimic their natural habitat.

Alike many other crops, wild populations of the vanilla crop and its relatives are under serious threat, as they are gradually pushed into extinction due to a combination of both biological traits

(e.g. mycorrhizal specificity) and human activities (e.g. habitat destruction and degradation, illegal collection, climate change) (Flanagan & Mosquera-Espinosa, 2016). As recommended by Roux-Cuvelier & Grisoni (2010), Azofeifa-Bolaños et al. (2014), Flanagan & Mosquera-Espinosa (2016), Goetssch et al. (2021), and Bramel & Frey (2021), a comprehensive plan at national, regional and international levels is required to safeguard *Vanilla*'s genetic resources and their use in future crop breeding. A combination of the following strategies has been proposed: *in situ* conservation of *Vanilla*'s natural habitat and its associated ecological interactions, *ex situ* conservation of the vanilla gene pool in gene banks, and *circa situm* community-based vanilla cultivation programs. The above-described SPASHA approach for the vanilla crop could enable the combination of two of these strategies in a single territory: (a) *in situ* protection of natural habitats comprising wild *Vanilla* populations, and (b) transformation of neighbouring, degraded lands into agroforestry systems that incorporate vanilla as a (additional) cash crop. By mimicking the complexity of their natural habitat, these agroforestry systems could serve as biological corridors for, amongst others, *Vanilla* pollinators and seed dispersers, while providing numerous ecosystem services as well as income generation (De Beenhouwer et al., 2013; Singh Tomar et al., 2021). Moreover, vanilla being an understorey forest species, we assume that these agroforestry systems could provide optimal growth conditions, and thus satisfying vanilla bean yields.

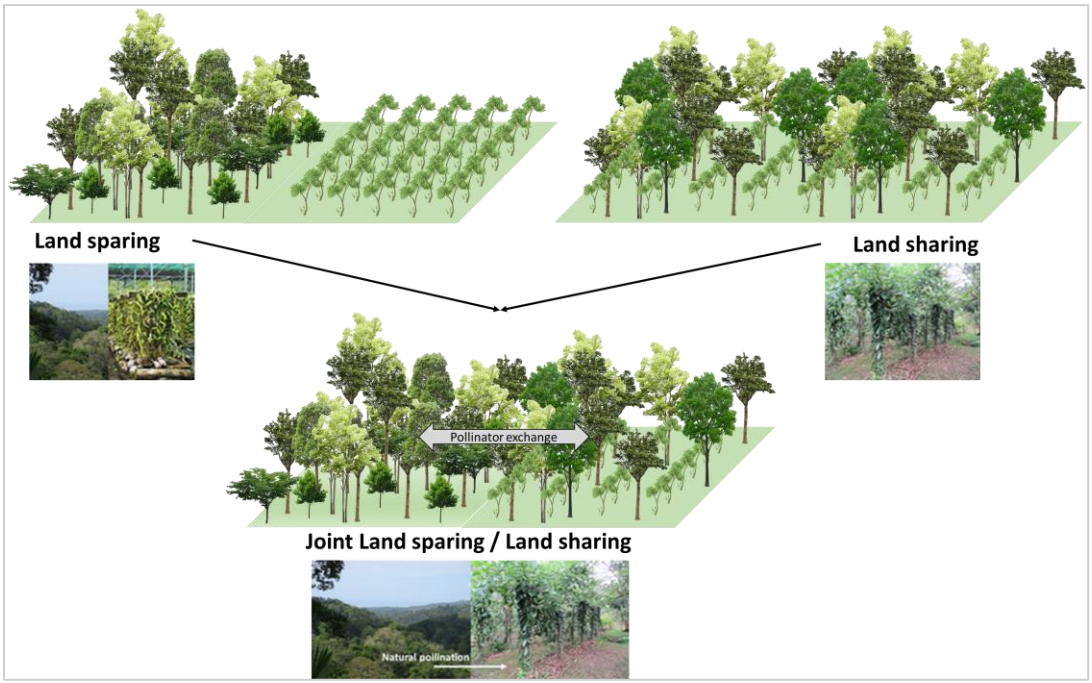

Fig. 1.3. *Visualisation of the combined land sparing and land sharing (SPASHA) approach proposed for the vanilla crop, whereby sustainable vanilla cultivation systems (e.g. agroforestry systems) may allow the exchange of essential ecological interactions between Vanilla plants and their environment, while they simultaneously provide a connection between the surrounding natural habitats necessary to protect the wild populations of Vanilla CWRs.*

1.4 SUPPLY CHAIN MANAGEMENT

1.4.1 Issues within global food supply chains

Increased globalization and a growing population greatly impact the sustainability of food supply chains (FSCs). As such, global food supply is often associated with economic injustice favouring trade over production and processing, high greenhouse gas emissions, pressure on natural resources, and poor working conditions in the regions of primary production and processing. Moreover, anonymity and disconnection due to large geographical and relational distances (Fonte, 2006; Wiskerke, 2009; Hua et al., 2018; Weber et al., 2020) vastly complicate trust building along the supply chain (Kneafsey et al., 2008; Lebel et al., 2008; Clapp, 2015). The trend of FSCs being dominated by retailers and characterized by unequal power distribution among the involved stakeholders, has led to the development of initiatives focusing on sustainable supply chain management (SSCM). SSCM, described as “the management of material, information and capital flows as well as cooperation among companies along the supply chain while taking goals from all three dimensions of sustainable development, i.e. economic, environmental and social, into account which are derived from customer and stakeholder requirements” (Seuring & Müller (2008), is built on the principles of partnerships, thereby ensuring the exchange of information necessary to optimize FSC efficiency (Hervani et al., 2022). There are two dimensions of collaboration that may exist: vertical collaboration between suppliers and customers, and horizontal collaboration between competitors and other actors, also defined as vertical and horizontal supply chain coordination, respectively.

As mentioned above, the distance within the supply chain can be both geographical and social. Geographical distance is the physical distance between the supply chain members, which is often large when talking about global food supply chains (Weber et al., 2020). Social or relational distance refers to the relationship between the supply chain members, whereby social proximity implies the capacity to establish a channel of communication between all parties involved. The latter involves the exchange of knowledge, value and meaning about the product and its provenance, production and consumption (Marsden et al., 2000; Watts et al., 2017). In addition to the number of intermediaries, it is therefore important to take into account the qualitative face of the relationship. Large distances entangle the integration of vertical and horizontal collaboration within the supply chain, which is why the concept of short food supply chains (SFSCs) emerged as a strategy to combat these difficulties and to improve resilience and sustainability (Weber et al., 2020). SFSCs represent an alternative type of food chain governance and organisation. Many are bottom-up initiatives in which producers and consumers, who are often passive and

subordinated participants in conventional global chains, become influential and active actors. Consumers are curious about the origins of their food and want to connect with the people that produce and process it (Kneafsey et al., 2008; Weber et al., 2020). Farmers on the other hand are getting increasingly organized through the formation of cooperatives at local or regional scales, strengthening their position within supply chains (Kumar et al., 2015). The reduction of geographical distances is often impossible due to the fact that some products can only be produced in certain geographical areas. In this context, focus should lay on shortening social distances via stakeholder coordination and the formation of stable and transparent cooperations (Haen & Requillart, 2014).

1.4.2 Driving sustainability within the vanilla supply chain

The vanilla supply chain is known to be a highly unstable one. Mainly two countries are monopolizing the worldwide production of vanilla (Madagascar – 2,975 tonnes, Indonesia – 2,306 tonnes) (Faostat, 2021). Madagascar is the dominating exporting country, followed by Papua New Guinea, while Indonesia exports less than 20 % of its total production (ITC, 2021). The main importing countries are the United States, followed by France, Germany, Canada, and the Netherlands, and they all re-export to other countries (ITC, 2021). Detailed information about revenues and price margins along the vanilla supply chain is, however, lacking – highly questioning the equity of revenue partitioning. Moreover, the monopoly of Madagascar in both vanilla bean production and export vastly reduces the resilience against unexpected events.

Vanilla is mostly grown by independent smallholder farmers, who bring their green beans to local markets. Here, the beans are bought by collectors to be sold to processors and refiners, who sell them to wholesalers or manufacturers. This sourcing model involves several intermediaries, making it often impossible to trace the origin of any given batch of vanilla beans (Fig. 1.4) (Ecott, 2004; Yoon et al., 2020). The large separation between farmers and exporters, with exporters receiving supplies from collectors gaining high profits by paying low prices to farmers (Munshi, 2020), exarcerbates the lack of trust among the involved stakeholders, and threatens the sector's traceability and stability. In brief, natural vanilla supply is characterized by boom and bust cycles, causing a harmful situation for both producers and buyers. For example, international market prices boomed in 2003 (> USD 300 / kg), drastically dropped in 2005 (< USD 50 / kg), and reached record highs (> USD 500 / kg) in 2017 and up to this day (Fig 1.5) (CBI, 2016; Rain, 2017; Teoh, 2019). When prices are high, global production increases as farmers perceive vanilla as an extremely high-value crop. These high prices, however, attract actors with opportunistic

behaviour, leading to theft problems and early harvests. As a result, the quality of the vanilla beans decreases, and the industry looks for alternatives such as synthetic vanilla. With the demand being lower than the supply, international market prices sink. The latter leads to lower prices paid at farmgate level, and an increased poverty among the vanilla farmers. Consequently, vanilla production decreases as only a few farmers continue to produce vanilla. Vanilla bean prices being rather unattractive, there is less theft and early picking, leading to better practices and thus higher vanilla bean quality. The industry switches again to natural vanilla, which increases the overall demand and results in supply shortages, causing prices to rise. Moreover, as it takes several years for vanilla farmers to reach production (generally three to four years after planting), this price volatility frequently causes market speculation (Thompson, 2017; Kunz et al., 2020). As a consequence of the boom and bust cycles, synthetic vanilla currently provides more than 90% of the vanilla used by the industry. Compared to the complex organoleptic profile of natural vanilla, synthetic vanilla only contains the compound vanillin, extracted from petroleum, spruce trees or the castor glands of beavers. Food businesses and their customers are aware of this fact, and the demand for natural vanilla is growing. Besides, companies are paying increasingly more attention to the social-ecological footprint of their raw material sourcing, and are searching for alternative vanilla sources that guarantee a sustainable provision and price stability (personal communication with food companies such as Verstegen Spices & Sauces B.V.).

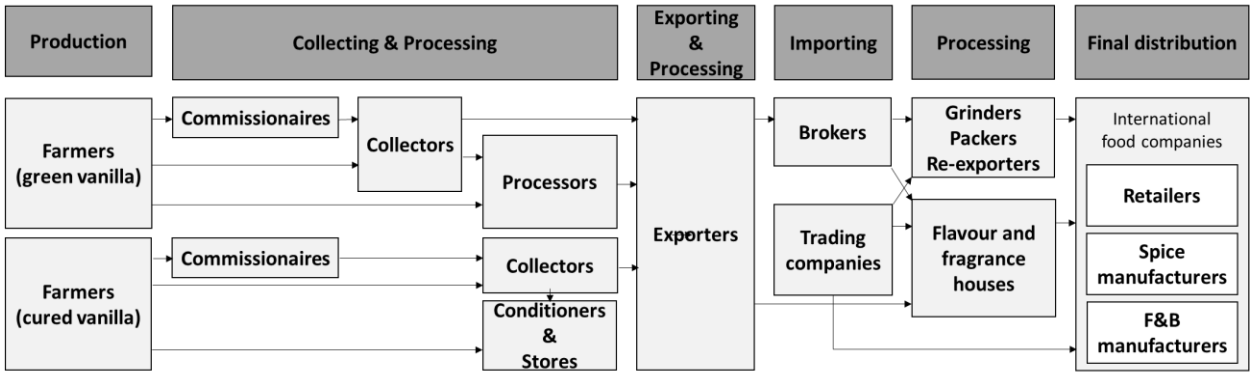

Fig. 1.4. *The general sourcing model of the vanilla supply chain (Adapted from Yoon et al., 2020).*

Supply chain coordination, i.e. horizontal coordination among farmers in agricultural cooperatives and vertical coordination in contracts between farmers and buyers (Vroegindewey & Hodbod, 2018; Ba et al., 2019), has the potential to foster transparency and efficiency in the vanilla supply chain. Moreover, the inclusion of small-scale farmers in the supply chain of high-value products such as vanilla, can have important effects on rural income mobility and poverty alleviation (Wang et al., 2021). There is, however, a need to improve the market demand for vanilla commodities

produced according to sustainability standards, and to strengthen market diversification. Herewith, private companies and end-consumers should give full support to sustainable agricultural practices and specialty markets. This can be done by improving farmers' market access, encouraging value-adding, stimulating the demand for sustainably produced vanilla beans, and promoting the use of a wider range of *Vanilla* varieties. Vanilla markets should be developed at local, national and international levels, and ensure a fair benefit share. In this context, an enhanced vertical and horizontal integration will be key for a successful transformation within the vanilla sector.

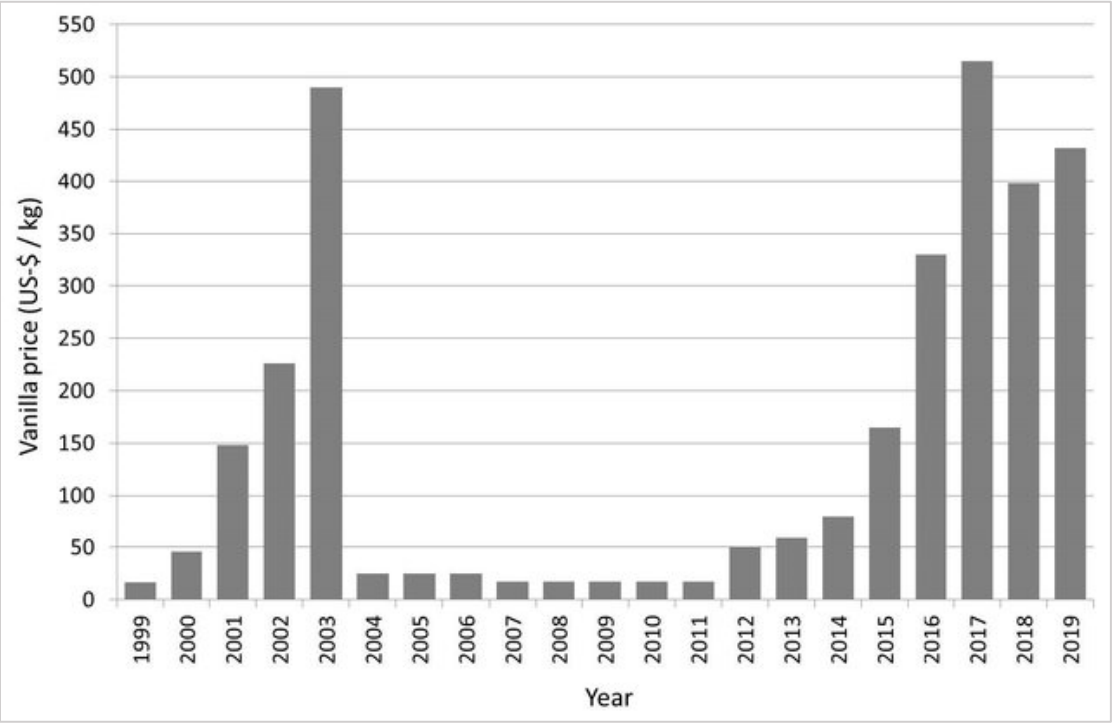

Fig. 1.5. *Historical boom and bust price cycles typical for the vanilla industry. Since the liberation of the vanilla market in the 1990s, vanilla prices have been highly volatile. In 2000, hurricanes damaged the majority of Madagascar's plantations, leading to an international price rise up to about USD 500 / kg in 2003. From 2004 onwards, there was an overabundance resulting in price drops. Due to the low revenues, vanilla farmers abandoned their plantations, leading to vanilla shortages in 2012-2013 and thus an increase in prices. Typhones in 2017 and 2018, however, caused a further rise in prices with records up to USD 500 / kg and more. Prices are expected to remain high for the coming years, as vanilla farmers are still recovering from the damages caused by these extreme weather events (Kunz et al., 2020).*

1.5 AIMS, OBJECTIVES AND THESIS OUTLINE

1.5.1 Aims and objectives

This PhD thesis **aims** at providing initial insights into possible measures to mitigate the identified production and socio-economic problems within the vanilla sector, by probing the potential use of *Vanilla* CWRs within a land sparing/sharing (SPASHA) approach that endeavours a joint effort of (a) the conservation of the natural habitat of *Vanilla* CWRs, and (b) the sustainable cultivation of vanilla in agroforestry systems surrounding these protected habitats, whereby the integration of promising *Vanilla* CWRs and their natural pollinators is highly encouraged. To formulate practical recommendations regarding the implementation of this joint approach of vanilla conservation and cultivation within the center of vanilla crop origin, we first require a better understanding of the natural occurrence, ecology, and agronomic potential of *Vanilla* CWRs, as well as the preferences of both farmers and consumers towards the proposed innovations. We focus on the Área de Conservación Osa (ACOSA), a biodiversity hotspot in southwest Costa Rica, where several *Vanilla* CWRs can be found and with land use policies that support the proposed approach.

We use an interdisciplinary approach that involves **four work packages** to accomplish abovementioned research aim, with each work package containing its own objective (Fig. 1.6). First, state-of-the-art species distribution modelling (SDM) techniques are applied to predict suitable areas for fragrant *Vanilla* CWRs (*Vanilla* subg. *Xanata* sect. *Xanata*) to occur, focusing on the ones found in Costa Rica. Subsequently, priority areas for *Vanilla* CWR conservation as well as areas of high potential for vanilla cultivation are identified to be incorporated into our case-specific *Vanilla* SPASHA approach. Second, an in-depth comprehension of *Vanilla* pollination mechanisms is required to provide information on the potential exchange of natural *Vanilla* pollinators between forest and farmland, as proposed by the SPASHA approach. Pollination is a crucial step to ensure vanilla bean production, but current cultivation systems fail to integrate natural pollinators and suffer from high costs associated with the labour-intensive manual pollination technique. Considering the lack of knowledge, the pollinators and associated pollination mechanisms are to be identified, and natural reproductive success to be examined. Third, plant performance, and more specifically the survival, vitality, growth, production and aromatic potential, is compared among *Vanilla* species (i.e. CWRs and commercially-used species) and land use system (i.e. (re)forested area and cacao agroforestry) to evaluate the cultivation potential within systems that may function as biological corridors. Combining the information attained from these three work packages, recommendations can be provided to develop agro-ecological vanilla cultivation systems that incorporate *Vanilla* CWRs and natural

pollinators. Last, socio-economic methods will be used to gauge the interest and preferences among farmers towards the proposed strategy, and to better understand their perspectives on the use of *Vanilla* CWRs, natural pollinators, sustainable agroforestry systems, and supply chain organization.

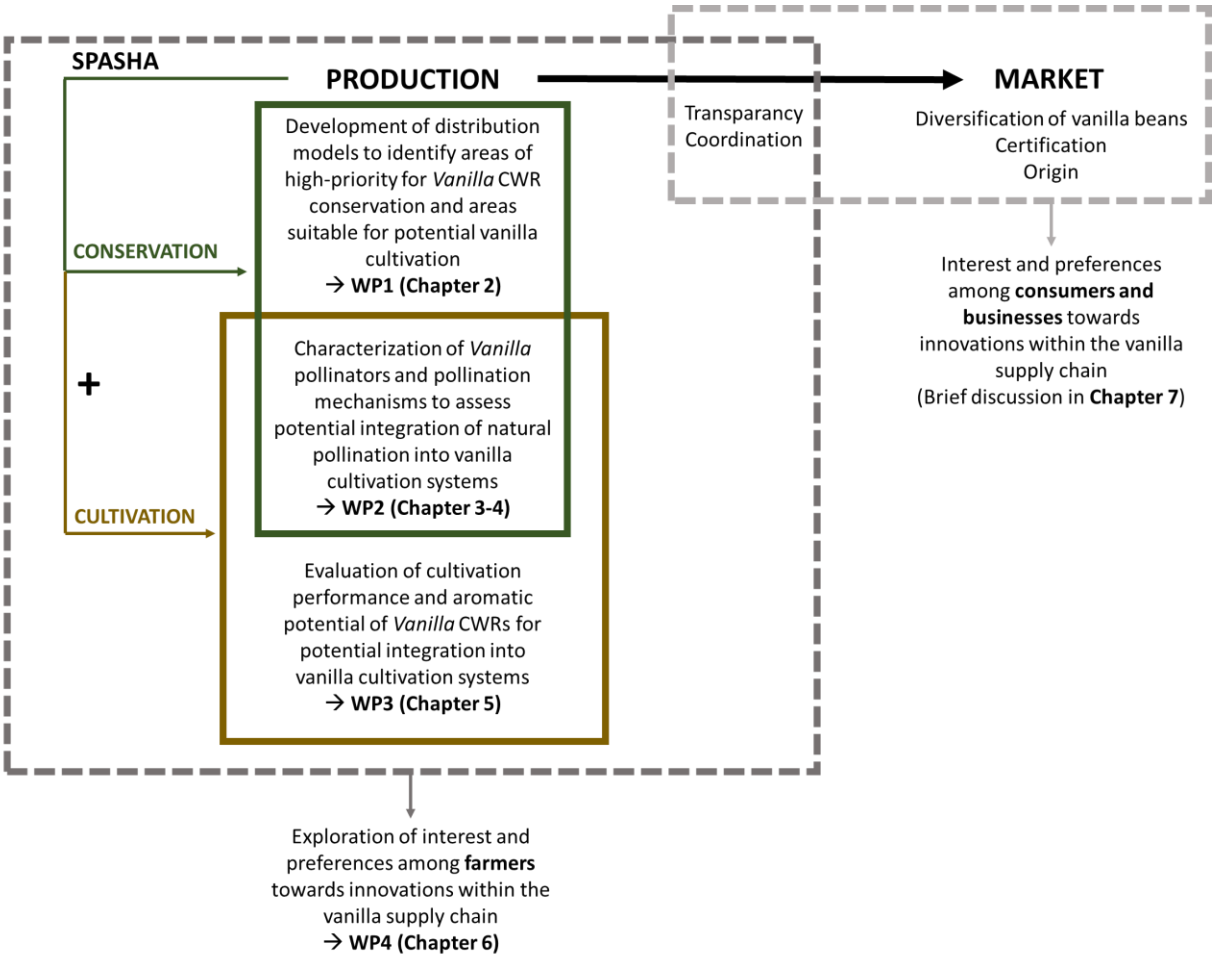

Fig. 1.6. *Schematic overview of the four work packages, each with its own objective.*

1.5.2 Thesis outline

In the following chapters we address the abovementioned objectives related to each of the four work packages

In **Chapter 2** we model the distribution of *Vanilla* CWRs, known to occur in Costa Rica, by applying ensemble SDMs, i.e. a robust SDM technique that combines different modelling algorithms. Occurrence data of the 14 *Vanilla* species naturally growing in Costa Rica are collected along their native growing ranges across the Neotropics, using a combination of herbaria records, scientific articles, reports, and personal forest inventories. The dataset of observed

presence locations is combined with climate, soil and topographic data to predict suitable areas for *Vanilla* CWRs to occur, i.e. areas that satisfy the ecological niche requirements of these species. Species distribution maps are developed for Costa Rica and overlaid with both land use and biological corridor maps to identify areas to be dedicated to *Vanilla* CWR conservation as well as promising vanilla cultivation areas, with the latter including compelling *Vanilla* CWRs. By recognizing these areas, we are able to provide spatially-explicit recommendations regarding the implementation of the case-specific joint land sparing/land sharing strategy for the vanilla crop in our study region ACOSA.

In **Chapters 3 and 4** we identify the pollinator species and pollination mechanisms of *Vanilla* CWRs occurring in our study region ACOSA. A combination of observational, phytochemical, and morphological studies is applied. We use camera observations to differentiate effective pollinators from casual visitors by examining the behaviours of the floral visitors. Floral visitors are caught, identified at species level, and their morphological traits are compared with flower traits to recognize a potential morphological fit. Reproductive success is measured by calculating the fruit set at the end of each of the observed flowering periods. Additionally, phytochemical data on the presence or absence of floral rewards such as nectar and volatile compounds (VOCs) allows the identification of specific pollinator attraction mechanisms performed by the flowers. The obtained information provides a better understanding of vanilla pollination mechanisms, resulting in the development of guidelines for the potential integration of natural pollinators within vanilla cultivation systems.

Chapter 5 explores the early-growth performance and market potential of *Vanilla* CWRs. An experimental design consisting of twelve sites, six in cacao plantations and six in naturally regenerated secondary forests, is developed, in which six *Vanilla* species, two commercially-used species (i.e. the crop species *V. planifolia* and a hybrid between *V. planifolia* and the CWR *V. pompona*) and four CWRs (i.e. *V. hartii*, *V. odorata*, *V. pompona*, *V. trigonocarpa*), are integrated and monitored. Various traits related to the survival, vitality, growth, and production of the vanilla plants are measured for a period of two years, and compared among *Vanilla* species and land use system. Additionally measured environmental variables such as understorey light availability, microbial diversity and chemical soil parameters, are examined to further explain potential early-growth performance patterns. Furthermore, the aromatic profiles of the fruits of the *Vanilla* CWRs are compared with the ones of the commercially-used species. The gathered information allows us to provide insights into the cultivation and market potential of the observed *Vanilla* CWRs.

Chapter 6 seeks to identify the interest and preferences among farmers living in southwest Costa Rica (ACOSA) towards an agro-ecological vanilla cultivation approach, thereby providing insights on the potential of implementing the proposed innovative approach of vanilla farming. Household survey data is collected from 186 farmer families, representing contrasting social strata and living in areas with different land use policies (i.e. forest reserve, biological corridor, or outside former areas). A formal questionnaire is carried out, including modules on household demographics, land and non-land assets, farm production and marketing, employment and income, and social network. Furthermore, a discrete choice experiment (DCE), i.e. a statistical method used in the social science with applications in environmental and agricultural economics, is linked to the household survey, whereby the respondents are presented a series of choice cards with each card including a hypothetical situation, characterized by a set of cultivation, value chain and market conditions. For each choice card, respondents are asked to select the hypothetical situation they prefer most. The CE adds valuable information to elicit farmer's interest and preferences towards the proposed vanilla cultivation approach.

In **Chapter 7** we provide a general discussion on the outcomes generated from the four work packages, integrated with a brief overview of the main outcomes of an external questionnaire carried out with Flemish and Dutch end-consumers and food companies, in which the interest and preferences of these respondents towards new niche commodity markets for high-quality vanilla were explored. As such, conclusions are formulated regarding the potential development of the proposed vanilla cultivation approach within our study region. Finally, limitations and recommendations for continuous research on this exiting topic are provided.

1.6 STUDY REGION

Costa Rica offers favourable conditions to study possible applications of abovementioned innovations in the vanilla sector. The country is acknowledged as a leading tropical country in terms of forest restoration and sustainable development (Meyfroidt et al., 2010). In 1987, the government implemented the first Forestry Law and the establishment of economic incentives for forest restoration through Payments for Environmental Services (PES) (Steed, 2008), aiming to restore land that was converted for conventional agriculture. Thanks to this intervention, the forest cover has grown from 21 % in 1987 to more than 50 % currently, and restoration practices are continuing (Meyfroidt et al., 2010). Costa Rica lays within the native ranges of *Vanilla* and has a tradition of small-scale vanilla cultivation, focusing on the crop species *V. planifolia* and its hybrid with *Vanilla pompona* (Havkin-Frenkel & Belanger, 2018). There are more fragrant species

growing in the moist lowland rainforests of Costa Rica. Furthermore, the Costa Rican PES program, which is executed through the Fondo Nacional de Financiamiento Forestal (FONAFIFO) and the Sistema Nacional de Areas de Conservacion (SINAC), aims at protecting primary forest, allows secondary forest to develop sustainably, and promotes plantations to meet food demands. Therefore, Costa Rica serves as a promising area to explore the implementation potential of the *Vanilla* SPASHA approach, which can serve as a model system to be implemented in other countries of vanilla origin. We focus on the Área de Conservación Osa (ACOSA) in southwest Costa Rica (Fig. 1.7). ACOSA is an important endemism area and a biodiversity hotspot containing 2.5 % of the world's marine and terrestrial biodiversity (Kohlmann et al. 2010). The area holds wild populations of several *Vanilla* species (Soto Arenas & Dressler, 2010), and protected areas represent a third of its land surface, including two national parks (Corcovado, Piedras Blancas), the Golfo Dulce forest reserve, and the Golfito wildlife refuge. The average annual precipitation ranges from 2500 to 6000 mm, with a rainy season from May to November. The average annual temperature is 25 °C, with local variations as a result of topography and other geographic differences (Holdridge 1967, Kappelle et al. 2003). The dominant land uses in these areas are agriculture (oil palm and cacao) and cattle grazing.

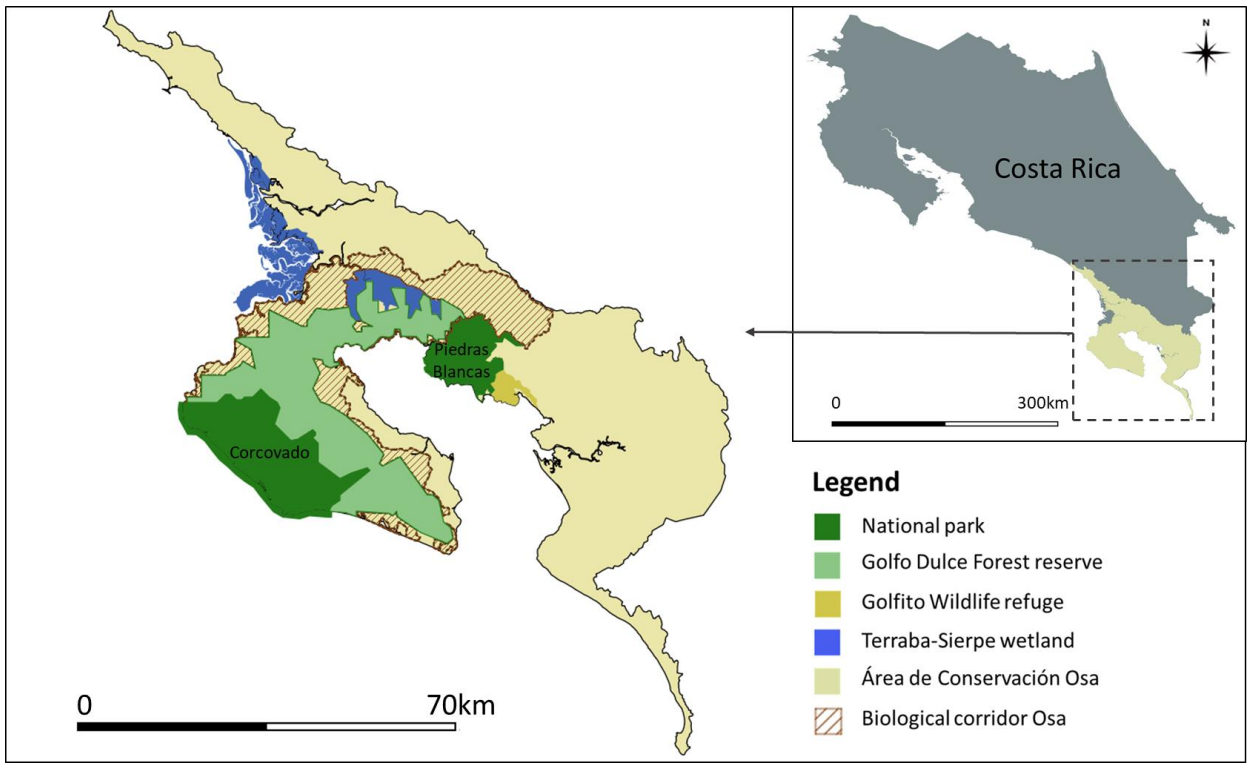

Fig. 1.7. *Map of our study area, Área de Conservación Osa (ACOSA), in southwest Costa Rica. Corcovado and Piedras Blancas are the two terrestrial national parks, and the Golfo Dulce reserve connects these two national parks. Golfito wildlife refuge borders Piedras Blancas national park, and the biological corridor Osa is part of the National Bio-Corridor Program (PNCB).*





2

Chapter 2

Vanilla distribution modelling for conservation and sustainable cultivation in a joint land sparing / sharing concept

Adapted from:

Watteyn, C., Fremout, T., Karremans, A., Pillco Huarcaya, R., Azofeifa Bolaños, J.B., Reubens, B., Muys, B. 2020. Vanilla distribution modelling for conservation and sustainable cultivation in a joint land sparing/ sharing context. Ecosphere, 11 (3), Art.No. e03056. doi: 10.1002/ecs2.3056

Photo: Searching for wild *Vanilla* populations in Piedras Blancas National Park, southwest Costa Rica (above). The typical landscape of ACOSA, consisting of forests surrounded by agricultural lands (below). © Charlotte Watteyn

2.1 SUMMARY

Vanilla, an expensive but popular spice used in many industries, faces problems related to its supply. Some of these problems are due to the fact that vanilla cultivation is based on clonal material of a single species, and is dominated by just a few countries located outside the native growing areas of *Vanilla* species producing the widely-appreciated fragrant vanilla beans (i.e. the Neotropics). Despite the economic importance of this crop, relatively little attention has been paid to its wild relatives, in particular with respect to their biology, ecology and potential use. We hypothesized that species distribution models (SDMs) can identify suitable areas for both the conservation and cultivation of *Vanilla* crop wild relatives (CWRs), following a joint land sparing/sharing (SPASHA) approach, and thereby offering alternative sourcing areas and production methods. This is the first study that explored the use of ensemble SDMs to provide applicable land use maps related to the conservation and sustainable cultivation of wild *Vanilla* species in Costa Rica. We focused on four fragrant *Vanilla* CWRs (*Vanilla* sect. *Xanata*), native to Costa Rica, to make land use policy recommendations for this country, and more specifically for our study region Área de Conservación Osa (ACOSA). The resulting distribution maps, with a mean AUC of 0.89, reflected the current distribution – ranging from unsuitable to suitable – of the modelled *Vanilla* species. Combining them with recent land use maps of ACOSA, we defined areas for (a) *Vanilla* CWR conservation, and (b) sustainable cultivation of *Vanilla* CWRs within agroforestry systems. These land use recommendations can now be integrated within the National Bio-Corridor Program (PNCB) that aims at making biological corridors economically productive, by proposing sustainable income generation for local communities living within these areas. Our approach can be applied to implement the SPASHA approach on other *Vanilla* CWRs and in additional regions across their native growing ranges, given the availability of land use maps and enough occurrence records to build accurate models.

2.2 INTRODUCTION

Vanilla is an expensive but crucial ingredient for many industries worldwide (Ramachandra Rao & Ravishankar, 2002; Havkin-Frenkel & Belanger, 2010; Cameron, 2011). It is extracted from the fruits, better known as beans or pods, of orchid vines belonging to the genus *Vanilla* Plum. ex Mill. (1754) (Soto Arenas & Dressler, 2010; Cameron, 2011). At present, the main production countries are Madagascar (3,227 tons/year), Indonesia (2,402 tons/year), and China (662 tons/year) (Faostat, 2021), all three located outside the native growing range of fragrant *Vanilla* species, which is the Neotropics (Purseglove, 1973; Bouetard et al*.,* 2010; Soto Arenas & Dressler 2010). Vanilla cultivation in these countries is therefore limited to the introduced species *Vanilla planifolia* Jacks. ex Andrews (Fouché & Jouve 1999; Lubinsky et al., 2006). Due to the fact that vanilla orchids are propagated in a vegetative way, the genetic diversity within the crop populations is relatively low, negatively affecting production resilience (Besse et al., 2004; Bory et al., 2010; Borbolla Pérez et al., 2016). Furthermore, the lack of natural pollinators in the abovementioned countries forces vanilla farmers to rely on hand-pollination, a labour-intensive and delicate activity (Villanueva-Viramontes et al*.*, 2017). As hand-pollination results in higher yields compared to natural pollination, this technique has been applied in commercial vanilla plantations worldwide, including those in the Neotropics (Cameron, 2011). Despite its high efficiency, manual pollination potentially leads to over-pollination, which stresses the plants and makes them less tolerant to abiotic and biotic stresses, such as droughts, temperature changes, and diseases caused by *Fusarium, Phytophtora, Glomerella*, amongst others (Havkin-Frenkel & Belanger 2018; Cameron, 2011; Ramos-Castellá et al*.*, 2016; Villanueva-Viramontes et al*.*, 2017). This downward spiral is exacerbated by the lack of genetic diversity in vanilla plantations.

The concentration of vanilla production in just a few countries also leads to a lack of resilience against extreme weather events, pathogens, political instability, theft, monopoly and processing errors (Havkin-Frenkel & Belanger, 2018). Notwithstanding the economic importance of this high-value cash crop, other fragrant *Vanilla* species besides *V. planifolia* have received relatively little attention from the scientific community, in particular with respect to their biology, ecology and potential use for crop improvement and breeding. More research is urgently needed to determine how we can preserve these wild species while also exploring the possibilities to cultivate them in sustainable production systems, thereby improving current systems based on the single species *V. planifolia* (Ramos-Castellá et al*.*, 2016). The lowland rainforests of the Neotropics are home to several fragrant *Vanilla* crop wild relatives (CWRs), which are wild species related to the vanilla crop (Bory et al., 2010; Flanagan & Mosquera-Espinosa, 2016). These CWRs, which are naturally

pollinated (Lubinsky et al., 2006; Pansarin & Pansarin, 2014, Anjos et al., 2016; Pansarin & Miranda, 2016), represent valuable sources of useful traits to improve current cultivation systems in terms of climate change adaptation, pathogen resistance, and production stability and quality (Meilleur & Hodgkin, 2004; Engels et al., 2006; Heywood et al., 2007). They could contribute to a possible solution for the current scarcity and skyrocketing prices of natural vanilla. However, like many orchids, most of these wild *Vanilla* species are under pressure, mainly due to deforestation, unsustainable collection from the wild and lack of conservation efforts (Verma et al., 2009; Herrera-Cabrera et al., 2017; Wegier et al., 2017). The genus *Vanilla* can therefore be considered as a group of flagship species for the effective protection of its CWRs, via an integrated approach of both conservation and sustainable cultivation (Flanagan & Mosquera-Espinosa, 2016; Herrera-Cabrera et al., 2018). We believe that this can be achieved through an innovative concept of joint land sparing / sharing (SPASHA) (Fig. 2.1). Land sparing sets aside some land for conservation and some for intensive cultivation, while land sharing combines the cultivation of crops with the conservation of native vegetation elements in the same areas (e.g. agroforestry) (Fischer et al, 2014; Phalan, 2018). The SPASHA approach proposed here combines these two strategies in a single territory, by protecting natural *Vanilla* populations and their pollinators inside forests, while using some of the neighbouring land for vanilla cultivation in agroforestry systems where the natural interactions are kept as intact as possible.

In this study, we return to the regions of origin of the fragrant *Vanilla* species, more specifically Costa Rica, and hypothesize that species distribution models (SDMs) can identify suitable areas for both the conservation and sustainable cultivation of *Vanilla* CWRs, enabling us to implement the proposed SPASHA approach. SDMs, also known as bioclimatic envelope models, ecological niche models and habitat suitability models, generate geographical maps of a species' environmental suitability. Thanks to the growing availability of freely accessible georeferenced species records (e.g. Global Biodiversity Information Facility, Botanical Information and Ecology Network) and environmental data (e.g. WorldClim, ISRIC), SDMs are now widely used in many ecological applications, such as the selection of priority areas for conservation, the prediction of climate change effects on species distribution ranges and the determination of species' invasion risks (Guisan & Zimmermann, 2000; Pearson & Dawson, 2003; Guisan & Thuiller, 2005; Phillips et al., 2006; Rodríguez et al., 2007; Elith et al., 2010; Jiménez-Alfaro et al., 2018).

We focus on four fragrant *Vanilla* CWRs (*V. hartii*, *V. odorata*, *V. pompona* and *V. trigonocarpa*), and provide spatially explicit recommendations for the application of the SPASHA approach within our study area Área de Conservación Osa (ACOSA) in southwest Costa Rica, where the main

economic activities are ecotourism and agriculture focusing on cattle and palm oil (Sierra & Russman, 2006; Hunt et al., 2015). Using the national biological corridor network of Costa Rica (SINAC, 2018), we define corridors with high vanilla suitability that can be reforested into productive agroforestry systems with vanilla as (one of) the cash crop(s). This promising strategy can be integrated in forest landscape restoration programs where agroforestry systems provide an income for local communities, while also connecting the surrounding protected forest fragments through a corridor network harbouring wild *Vanilla* populations. Our study demonstrates that SDMs are a useful technique to implement theoretical concepts, such as the proposed SPASHA approach, in land management and policy. We believe that *Vanilla* CWRs offer a high cultivation potential within sustainable agroforestry systems, and could contribute to the improvement of current crop production systems, restore degraded landscapes, and enhance socio-economic activities while satisfying global food demands. The proposed model can be used in other areas where *Vanilla* CWRs are present.

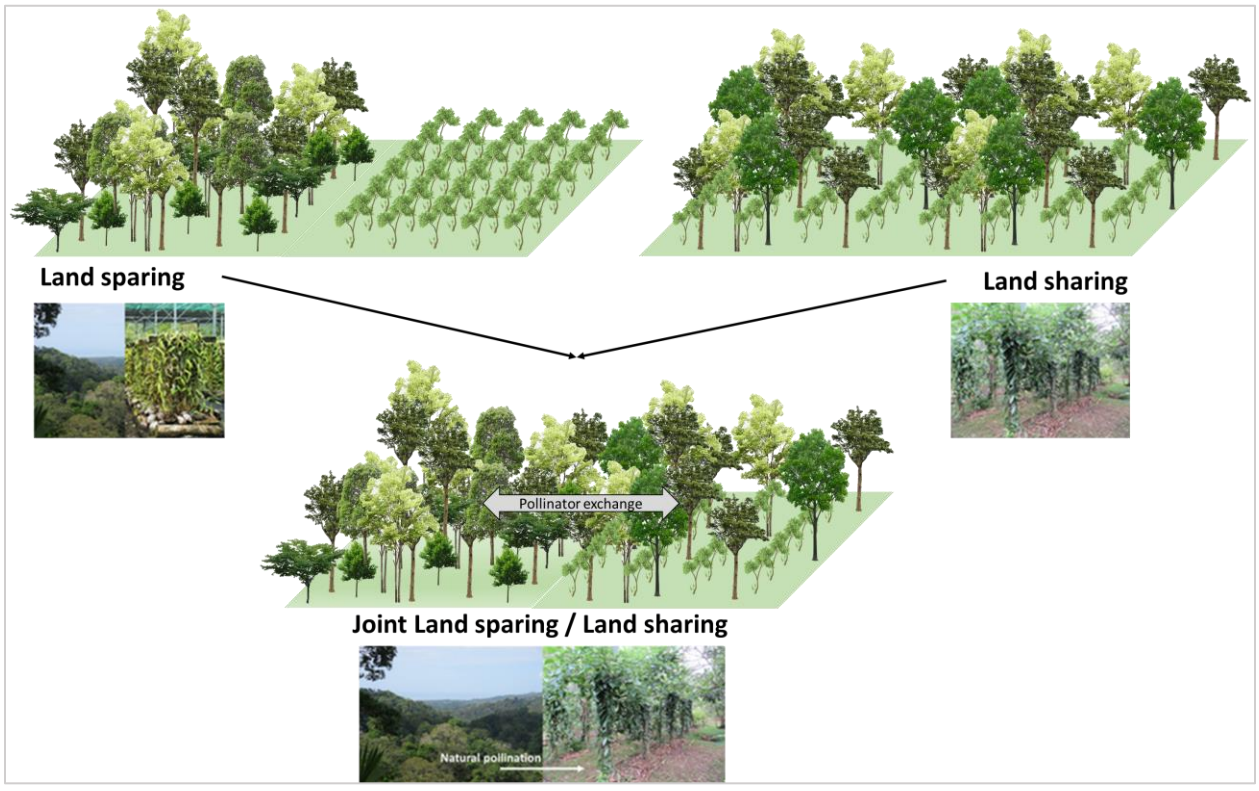

Fig. 2.1. *The concept of joint land sparing/sharing (SPASHA) explained for vanilla conservation and cultivation, allowing the exchange of important ecological interactions such as pollination between the forest and neighbouring vanilla agroforestry systems.*

2.3 MATERIAL AND METHODS

2.3.1 Study area

Costa Rica is located within the native growing ranges of several aromatic *Vanilla* species and has a tradition of small-scale vanilla cultivation, focusing on the commercial species *V. planifolia* and more recently a disease-resistant hybrid ((*V. planifolia* x *V. pompona*) x *V. planifolia*) (Quirós, 2010). Costa Rica's lowland rainforests harbour several wild *Vanilla* species of great interest for their potential in breeding programs, but understudied in terms of their biology and ecology (Azofeifa-Bolaños et al., 2017). An important area regarding the presence of *Vanilla* CWRs is the Área de Conservación Osa (ACOSA) in the southwest Costa Rica (Fig. 1.6). ACOSA is an important endemism area and a biodiversity hotspot containing 2.5 % of the world's marine and terrestrial biodiversity (Kohlmann et al., 2010). Protected areas represent a third of the total land surface of ACOSA. The average annual precipitation ranges from 2,500 to 6,000 mm, with most precipitation occurring during the rainy season from May to November. The average annual temperature is 25 °C, with local variations as a result of topography and other geographic differences (Holdridge, 1967; Kappelle et al., 2003). The dominant agricultural land uses in these areas are plantations of *Elaeis guinensis* (oil palm), *Tectona grandis* (teak) and *Gmelina arborea* (gmelina) and cattle grazing lands.

2.3.2 Study species

Vanilla Plum. ex Mill. is a pantropical genus belonging to the orchid family (Orchidaceae), comprising about 120 species of hemi-epiphytic and epiphytic climbing vines with branching stems (Soto Arenas & Dressler, 2010; Cameron, 2011). Within the Neotropical region approximately 62 *Vanilla* species are now recognized (Karremans et al., 2020), belonging to three distinct phylogenetic lineages, (a) the membranaceous clade that is sister to the other members of the genus, with 18 species (*Vanilla* subg. *Vanilla*); (b) six Antillean species that are phylogenetically related to African species (*Vanilla* subg. *Xanata* sect. *Tethya*); and (c) the aromatic clade (*Vanilla* subg. *Xanata* sect. *Xanata*) with 38 species. The commercial species *V. planifolia*, as well as other fragrant species fall within the latter clade (Soto Arenas & Dressler, 2010). Little is known about the exact distribution of these species. At present, 14 species have been reported for Costa Rica (*V. costaricensis, V. dressleri, V. hartii, V. helleri, V. inodora, V. insignis, V. karen-christianae, V. odorata, V. phaeantha, V. planifolia, V. pompona, V. sarapiquensis, V. sotoarenasii, V. trigonocarpa*), in addition to the hybrid species mentioned above (Soto Arenas & Dressler 2010, Azofeifa-Bolaños et al. 2017, Karremans et al., 2020).

Seven of these species (*V. dressleri, V. hartii, V. helleri*, V*. karen-christianae, V. odorata, V. pompona, V. trigonocarpa*) are found within our study region ACOSA (based on forest inventories carried out within ACOSA), all belonging to the aromatic clade.

2.3.3 Species distribution modelling

We used an ensemble modelling approach (i.e. a combination of different modelling algorithms) to model the distribution of *Vanilla* CWRs under current climate conditions and to identify suitable areas for vanilla conservation and cultivation. The ensemble modelling was implemented in the R package BiodiversityR (Kindt, 2018), using the following twelve commonly used distribution modelling algorithms: Maxent (MAXENT), random forests (RF), support vector machines (SVM), flexible discriminant analysis (FDA), multivariate adaptive regression splines (MGCV), domain algorithm (DOMAIN), and a stepwise and non-stepwise implementation of boosted regression trees (BRT), generalized linear models (GLM) and generalized additive models (GAM). These algorithms are presence-absence algorithms, with the exception of Maxent (presence-background) and DOMAIN (presence-only). Both suitability and presence-absence maps were generated for Costa Rica and cropped to the extent of our study region ACOSA. This is further explained in the paragraph “Distribution modelling”.

Presence data and modelling extent

A dataset of georeferenced presence locations of *Vanilla* species occurring in Costa Rica was compiled from different sources, including records from online databases such as the Global Information Facility (GBIF) (gbif.org, 2019), the Botanical Information and Ecology Network (BIEN) (Enquist et al*.*, 2016) and Tropicos (tropicos.org 2019), scientific articles and reports (e.g. Soto Arenas & Dressler 2010; Azofeifa-Bolaños et al., 2017; Karremans & Lehmann, 2018), and forest inventories carried out within our study area and the rest of Costa Rica by ourselves, local universities (Universidad de Costa Rica and Universidad Nacional de Costa Rica) and partner organizations such as Osa Conservation and Ministerio de Ambiente y Energia – Sistema Nacional de Áreas de Conservación (MINAE-SINAC). When using SDMs, the extent of the modelling range is of particular importance. It has been recommended to use occurrence data from the complete distribution range of the species. Two important reasons are: (a) when taking a subset, it likely does not include the full environmental variation under which a species is occurring and (b) many species are rare and are represented by just a few records (Reddy & Dávalos, 2003; Schulman et al., 2007; Beaumont et al., 2008; Hortal, 2008; Sánchez-Fernández et al., 2011; Barbet-Massin et al*.*, 2012; Raes, 2012; Gomes et al., 2018). Completely opposite,

others have suggested to select a restricted distribution range because of the occurrence of subspecies within for example geographically isolated areas, and pooling these subspecies for analyses might mask biologically relevant spatial variation within the distribution range of a single species (Gonzalez et al., 2011; Gomes et al., 2018). We chose to use the Neotropics (Mexico towards south Brazil) as our extent for occurrence to prevent the aforementioned deficiencies associated with a narrow geographical and thus environmental selection, and especially because of the scarcity of available georeferenced data on *Vanilla* species (Pupulin & Karremans, 2017).

The compiled data was cleaned by removing all records with missing locality information, and all records with coordinates located in the ocean, coordinates with latitude or longitude equal to zero, or with latitude equal to longitude (Boyle & Smith, 2010; Maldonado et al., 2015; Zizka & Antonelli, 2015). Four *Vanilla* CWRs (*V. hartii*, *V. odorata*, *V. pompona* and *V. trigonocarpa*) had more than 30 species occurrence records, which we considered enough to build accurate distribution models (Wisz et al., 2008; Mateo et al., 2010; van Proosdij et al., 2016), and all four of them are present within our study area ACOSA. The SDMs were carried out with minimum half of the presence and absence data within Costa Rica. If there were more data outside Costa Rica, a random subsample was taken outside Costa Rica with the same amount of data as inside.

Background / pseudo-absence data and target-group background approach

In addition to the presence data, all modelling algorithms used in this study required the input of absence or pseudo-absence data except for MAXENT (presence/background) and DOMAIN (presence-only). Pseudo-absence and background points were selected using the target group approach (Phillips et al., 2009), a method that has been used to improve the predictive performance of SDMs in the presence of spatially biased presence data (Phillips et al., 2009; Elith et al., 2010; Gomes et al., 2018). In the target group approach, pseudo-absence or background records are selected from grid cells with presence data of species that belong to a similar group of species as the target species, under the assumption that these locations reflect a similar bias as the sampling bias of the target species (Phillips et al., 2009).

In our case, the target group grid was constructed using the occurrence records of all hemi-epiphyte and liana species growing in Costa Rica (species list derived from tropicos.org). Presence data of these target group species was derived from GBIF and BIEN, and cleaned using the same method as explained before. Background points were randomly selected from this grid. The pseudo-absence points were generated by selecting grid cells that did not contain records of the *Vanilla* species being modelled, and that had records of at least 20 other species. In this way, the pseudo-absence points were assumed to be relatively close to real absence points.

Predictor variables

Data of 19 bioclimatic variables with a 1km resolution was derived from the WorldClim database (Hijmans et al., 2005). Furthermore, we selected eight continuous soil variables with a 250m resolution from the ISRIC SoilGrids250m database (Hengl et al., 2014), derived five topographic variables with a 250m resolution from the Global Multi-resolution Terrain Elevation Data 2010 (GMTED2010) (Danielson & Gesch, 2011; Amatulli et al., 2018) and extracted cloud cover data with a 1km resolution from the global remote sensing-derived cloud database (Wilson & Jetz, 2016). We used the variance inflation factor (VIF) to avoid problems with multicollinearity of the predictor variables, using a stepwise approach that results in a set of variables that all have a VIF lower than 10 (García et al., 2015). Our final set contained nineteen predictor variables: mean diurnal range, maximum temperature of warmest month, mean temperature of coldest quarter, annual precipitation, precipitation seasonality, precipitation of warmest quarter, precipitation of coldest quarter, available water capacity, cation exchange capacity, clay content, coarse fragments, organic carbon content, pH index measured in water solution, silt content, aspect, slope, topographic position index and cloud cover. Variables with a higher resolution of 250m were resampled so all predictor variables had a spatial resolution of 250m.

Distribution modelling

The SDMs were evaluated using the area under the receiver operating characteristic curve (AUC) measure. The AUC measure is a threshold-independent statistic used in ecological modelling to assess the capacity of a model to discriminate between positive (presences) and negative (absences) instances (Phillips et al., 2006). The use of AUC has been criticized mainly because the increase of the geographical extent, and thereby the environmental extent, in which pseudo-absences are selected, causes an overestimation of AUC values (Lobo et al., 2010). To avoid this, we selected pseudo-absence and background points within a convex hull around the presence records, extended with a buffer of 10% of the longest distance between presence records. We cross-validated the models with presence and absence data arranged in spatially independent blocks, using the 'blockCV' package (Valavi et al., 2019). Presence and absence data were divided into 100 km-wide squared blocks arranged in 8 cross-validation folds. If the distribution range of a species was not large enough to use 8 blocks, a lower amount of folds was used. Using the ensemble.tune function of the BiodiversityR package, the weights of the different algorithms used in the ensemble model were optimized during each cross-validation run, and the average of these optimized weights was used to make the suitability predictions of the final ensemble model. Prior to making these predictions, the algorithms included in the ensemble

model were calibrated again with all available occurrence data. To convert the continuous suitability predictions to presence-absence maps, the sensitivity-specificity criteria was used, one of the two methods recommended by Jiménez-Valverde and Lobo (2007), whereby the difference between sensitivity (true predicted presences) and specificity (true predicted absences) was calculated for the same 100 threshold values and the one which minimized that difference was selected. The resulting presence-absence maps were masked by the aforementioned extended convex hull to exclude areas without any occurrence records.

Variable importance and response curves

We evaluated the importance of each predictor variable and visualized the response of the species to the three most important predictor variables using the evaluation strip method (Elith et al., 2005). The importance of the different predictor variables in the ensemble model predictions was calculated using a randomization procedure that measures the correlation between the original predicted suitability values and the predicted values where the variable under investigation is randomly permutated. If the contribution of a variable to the suitability predictions is high, the suitability predictions are more affected by the permutation of the variable in question, resulting in a lower correlation. Therefore, '1 – correlation' was used as a measure of variable importance (Guisan et al., 2017).

Identifying vanilla conservation and cultivation areas - SPASHA approach

Maps showing the national biological corridor network of Costa Rica (SINAC, 2018) were overlaid with the generated presence-absence (1 / 0 values) maps to identify corridors with potential for the implementation of the SPASHA approach (i.e. areas with value 1 on the presence-absence maps). Focus for further specific land use recommendations was laid on the Osa corridor and its surroundings within our study region ACOSA. The presence-absence maps were cropped to the extent of ACOSA and overlaid with a recent land use map of this area (Shrestha et al., 2019) and a map depicting the protected areas (SINAC, 2018). Based on these final maps, we could make recommendations regarding land use management related to the SPASHA approach focusing on *Vanilla* CWRs, and based on the parameters given in Table 2.1. For example, if an area shows to be suitable for a certain *Vanilla* species to occur based on the distribution modelling, but is currently a forest patch laying outside a protected zone, it is seen as a priority area for vanilla conservation.

Table 2.1. *Overview of land use recommendations based on the final maps indicating unsuitable or suitable areas for vanilla cultivation, and overlaid with recent land use maps of the study area and the corresponding current conservation status.*

Suitability	Current land use	Current conservation status	Recommendation
High	Forest	Protected	No priority conservation area, continued vanilla conservation
High	Forest	Not protected	Priority area for vanilla conservation
High	Plantation (palm, cacao, timber)	N.A.	Vanilla cultivation in agroforestry systems
High	Grassland	N.A.	Vanilla cultivation in agroforestry systems
Low	Forest, plantation, grassland	Protected or not	No vanilla conservation or cultivation required
N.A.	Waterbody, mangrove, wetland	Protected or not	No vanilla conservation or cultivation required
N.A.	Urban	N.A.	No vanilla conservation or cultivation required

All analyses were performed with R (version 3.4). We used the package BiodiversityR for ensemble suitability modelling and the packages ggmap, ggplot2, rgdal, rgeos, maptools, dplyr, tmap, mapview, leaflet and raster for mapping operations.

2.4 RESULTS

2.4.1 Species distribution modelling

The ensemble SDMs had a mean AUC value of 0.89. The mean AUC values for the four modelled species (*V. odorata*, *V. pompona*, *V. hartii*, *V. trigonocarpa*) were respectively 0.86, 0.84, 0.94 and 0.93. Overall, the ensemble model, support vector machines (SVM) and maximum entropy model (MaxEnt) showed the highest performance among all species, as shown in Table 2.2. For all species, the ensemble model performed better than the individual algorithms. The most important predictor variables explaining the distribution of these four *Vanilla* species are described in more detail below. The suitability maps representing areas with values ranging from 0 (unsuitable) to 1000 (highly suitable) and the AUC values of the individual modelling algorithms can be found in Appendix I (Table A1.1-1.4 and Fig. A1.1).

Table 2.3 gives an overview of the contribution, with values ranging from 0 to 1, of each individual predictor variable included in the SDMs as well as the total contribution of soil, precipitation, temperature and topography separately and the overall contribution of all predictor variables together.

Table 2.2. *Overview of the mean AUC values for the four Vanilla species and the AUC values of the individual algorithms with the highest and lowest performance.*

AUC values	***V. odorata***	***V. pompona***	***V. hartii***	***V. trigonocarpa***
Mean AUC	0.86	0.84	0.94	0.93
Minimum AUC	0.75 (GBMSTEP)	0.70 (DOMAIN)	0.69 (EARTH)	0.76 (GLMNET)
Maximum AUC	0.91 (ENSEMBLE)	0.89 (ENSEMBLE)	0.95 (ENSEMBLE)	0.93 (ENSEMBLE)
	0.86 (MAXENT)	0.85 (SVM)	0.91 (SVM)	0.91 (MAXENT)

Table 2.3. *Overview of the contributions, with values ranging from 0 to 1, of the individual predictor variables included in the SDMs towards the distribution of the four Vanilla species, and the total contribution of soil, precipitation, temperature and topography for each Vanilla species.*

Predictor variables	***V. odorata***	***V. pompona***	***V. hartii***	***V. trigonocarpa***
Available water capacity	0.018	0.210	0.061	0.046
Cation exchange capacity	0.010	0.027	0.018	0.002
Clay content	0.068	0.004	0.071	0.011
Coarse fragments	0.049	0.006	0.082	0.004
Organic carbon content	0.006	0.018	0.019	0.006
pH (water solution)	0.005	0.023	0.007	0.012
Silt content	0.001	0.016	0.024	0.001
Soil (total)	*0.157*	*0.304*	*0.282*	*0.082*
Annual precipitation	0.313	0.003	0.069	0.124
Precipitation of coldest quarter	0.005	0.045	0.056	0.053
Precipitation of warmest quarter	0.003	0.019	0.285	0.044
Precipitation seasonality	0.237	0.003	0.075	0.010
Precipitation (total)	*0.558*	*0.070*	*0.486*	*0.230*
Max. temperature of warmest month	0.016	0.067	0.045	0.186
Mean diurnal range	0.011	0.002	0.115	0.006
Mean temperature of coldest quarter	0.015	0.053	0.040	0.054
Temperature (total)	*0.042*	*0.122*	*0.201*	*0.246*
Aspect (eastness)	0.000	0.001	0.012	0.004
Aspect (northness)	0.001	0.003	0.001	0.004
Slope	0.012	0.046	0.002	0.034
Topographic position index	0.017	0.004	0.010	0.001
Topography (total)	*0.030*	*0.053*	*0.026*	*0.043*
TOTAL	***0.787***	***0.550***	***0.994***	***0.602***

The most important predictor variables for *V. odorata* were the precipitation variables annual precipitation (0.31) and precipitation seasonality (0.24). The soil variables clay content (0.07) and coarse fragments (0.05) followed, but played a smaller role. The main variable explaining the distribution of *V. pompona* was the soil variable available water capacity (0.21), followed by maximum temperature of the warmest month (0.07) and mean temperature of the coldest quarter (0.05), but to a lower extent. The key predictor variables for *V. hartii* were precipitation of the warmest quarter (0.29) and mean diurnal range (0.12). Additionally, soil variables such as coarse fragments (0.08), soil clay content (0.07), available water capacity (0.06) were also fairly contributing to its distribution. The most important variables for *V. trigonocarpa* were maximum temperature of the warmest month (0.19) and annual precipitation (0.12). On average, precipitation and/or temperature variables (climate) contributed most to the distribution of the species *V. odorata*, *V. hartii* and *V. trigonocarpa* whereas soil and also precipitation were more important for *V. pompona*. Overall, the predictor variables included in the model explained a considerable proportion of the *Vanilla* species distribution. All the included predictor variables together contributed for 0.79, 0.55, 0.99 and 0.60 in the final distribution maps of respectively *V. odorata*, *V. pompona*, *V. hartii* and *V. trigonocarpa*.

In general, the mountainous areas (> 1000 m) are less suitable or unsuitable for the studied *Vanilla* species. The lowland areas (0 to 500 m) showed highest suitability, with some variations among the species, which depended on local or regional climate, soil and topographic variables as mentioned above. For example, the drier areas in northwest Costa Rica are suitable for *V. pompona*, but not for the other species. Both Pacific and Caribbean coast lines have suitable environmental conditions for the four *Vanilla* species to occur, but *V. hartii* and *V. trignocarpa* have a more restricted distribution compared to *V. odorata* and *V. pompona*, where *V. pompona* seems to be the most robust species with the widest potential distribution along Costa Rica.

2.4.2 Maps for priority conservation and sustainable cultivation of *Vanilla* CWRs

For each of the four *Vanilla* species, the modelled presence-absence maps, derived from the suitability maps, were overlaid with the national biological corridor map of Costa Rica to identify the corridors with suitability for the implementation of the SPASHA approach. This is illustrated for the species *V. odorata* (Fig. 2.2) whereas the results for the other *Vanilla* species can be found in Appendix I (Fig. A1.2). Overall, the biological corridor Osa and its surrounding areas within our study region ACOSA contain suitable areas for all four *Vanilla* species to occur.

After cropping these maps to the extent of our study region ACOSA and overlaying them with a recent land use map and a map of the protected areas of ACOSA, a final map was created for each species. The final maps highlight areas for conservation and areas recommended for sustainable cultivation, focusing on the biological corridor Osa as priority area but also its surrounding areas. Such a final map is illustrated for *V. odorata* (Fig. 2.3) and land use policy recommendations can be made using this map together with Table 2.1. The maps for the other species can be found in Appendix I (Fig. A1.3). In general, green areas are forests suitable for vanilla to occur, giving these areas a recommended conservation status, but the priority further depends on the location (in- or outside protected areas). For example, areas classified green located within the national parks are protected and thus of less concern, whereas those located outside the national parks should receive high priority regarding vanilla conservation, especially the Osa corridor as part of the PNCB. The brown and yellow areas are plantations and grazing lands respectively, recommended for the implementation of sustainable agroforestry systems (AFS) with vanilla as a cash crop, especially in areas where natural vanilla populations potentially occur in the nearby forests. The white areas are unsuitable for vanilla to occur, so with no recommendations regarding vanilla conservation or cultivation.

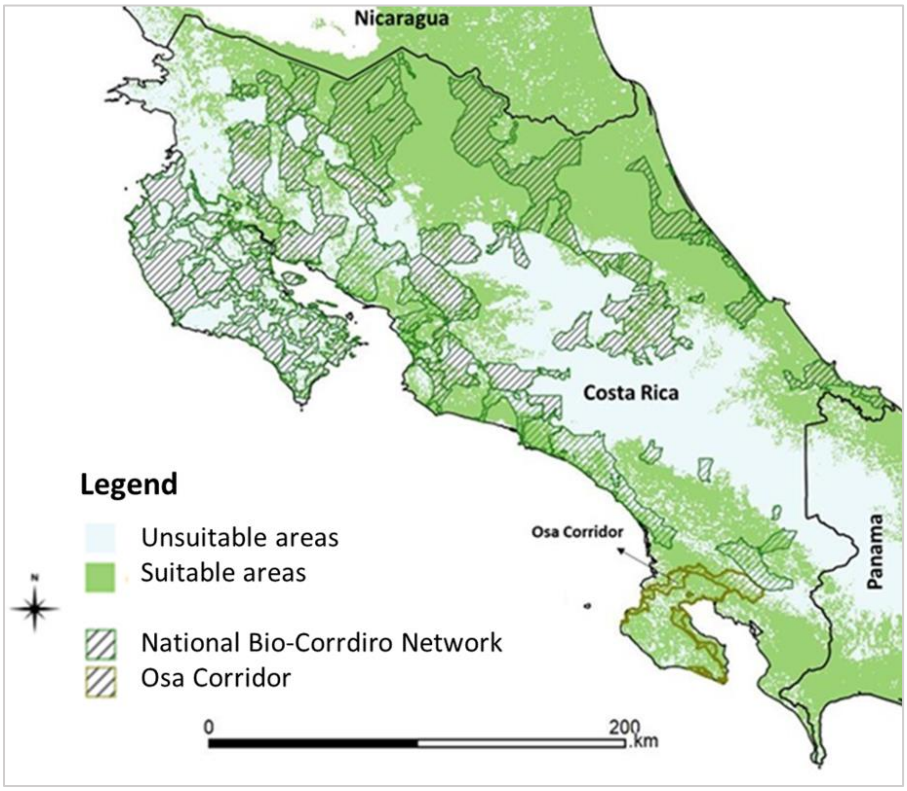

Fig. 2.2. *Map displaying the areas that are expected to be suitable for V. odorata, with a focus on our study region ACOSA and the corridor Osa. The light green areas are the areas suitable for the species in question to occur.*

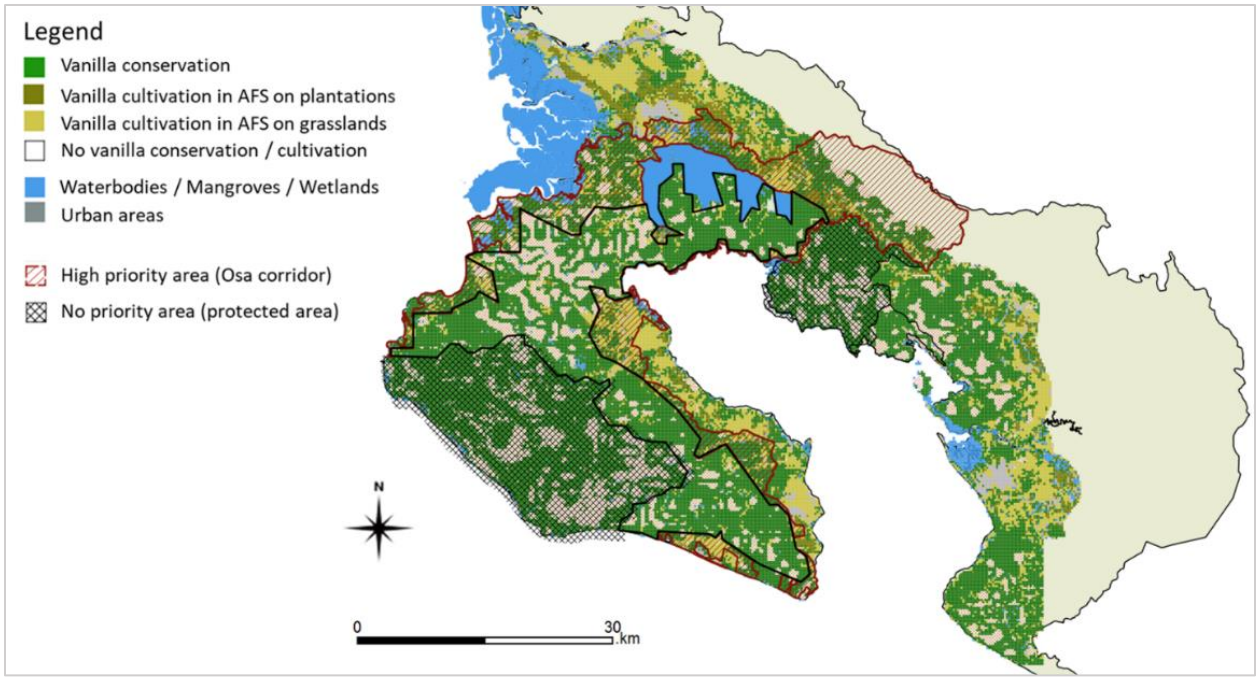

Fig. 2.3. *Map highlighting areas for conservation and sustainable cultivation of V. odorata within the ACOSA region. The green areas are forests suitable for vanilla to occur, giving these areas a vanilla conservation status, but the priority further depends on the location (in- or outside protected areas such as national parks). The brown and yellow areas are respectively plantations and grazing lands recommended for the implementation of sustainable agroforestry systems (AFS) with vanilla as a cash crop, especially in areas where natural vanilla populations (potentially can) occur in the nearby forests. The white areas are unsuitable areas for vanilla to occur, so with no recommendations regarding vanilla conservation or cultivation.*

2.5 DISCUSSION

2.5.1 Vanilla distribution maps

This is the first study that explored the use of species distribution modelling to identify priority areas for the conservation and sustainable cultivation of *Vanilla* CWRs. The created distribution maps for Costa Rica had a mean AUC value of 0.86, 0.84, 0.94 and 0.93 for respectively the species *V. odorata*, *V. pompona*, *V. hartii* and *V. trigonocarpa*, which we considered highly satisfactory to use for further application of the *Vanilla* SPASHA approach. We made land use recommendations for our study region ACOSA, using a recent land use map and protected areas map. The final maps (Fig. 2.3 for *V. odorata*, Fig. A1.3 for the other species) can now be used to implement our recommendations within the corridor Osa and its surrounding areas.

The SDMs showed that most of the lowland (< 500 m) rainforests along the Pacific and the Caribbean coast of Costa Rica possess suitable environmental conditions for all modelled species. The drier area in the northwest of Costa Rica seems to be only suitable for *V. pompona*,

which might be the result of its thicker stem and leaves giving it a higher tolerance towards droughts. The central mountainous region is, as expected, not favourable, probably due to unsuitable climate conditions at higher elevations (e.g. colder temperatures and a less pronounced dry season needed for flowering). The predictor variables included in the SDMs contributed to a noteworthy percentage of the distribution of all *Vanilla* species. Other factors, not included in the model, are most likely the presence of mycorrhiza and other soil microbiota. It is well-known that orchids have an important relationship with root fungi. A single orchid species can be associated with several mycorrhizal fungi, with different functional consequences for the plant (Ruibal et al., 2017). This also applies for *Vanilla* species, where it has been shown that different fungi species (e.g. *Ceratobasidium*, *Tulasnella*) play an important role in seed germination and plant growth (Porras-Alfaro et al., 2008, Bayman 2008; Cameron, 2011; Flanagan & Mosquera-Espinosa, 2016; González-Chávez et al., 2018). There are also still a lot of questions regarding vanilla pollination and seed dispersal, which could be important factors in explaining the distribution patterns of these *Vanilla* CWRs.

The set of *Vanilla* species initially considered in this study included all the fragrant CWRs native to Costa Rica and contained presence data among their whole natural distribution range within the Neotropics, but most of them could not be included in the final analysis because not enough observations were available to construct accurate models. We defined a minimum sample size of 30 observations (based on amongst others Stockwell & Peterson, 2002), proven to be sufficient to perform reliable SDMs. Nevertheless, the lack of presence data confirms the difficulty but also the importance of inventories and developing SDMs for these endangered species, and stresses the need for more data collection on wild *Vanilla* species, especially considering its economic value and endangered status.

2.5.2 Joint land sparing/sharing for the vanilla crop

We provide spatially-explicit recommendations for (a) the conservation of the four studied *Vanilla* CWRs, by protecting the forests that show high suitability for these species to naturally occur, and (b) the cultivation of these species within sustainable farming systems around these forests, i.e. in areas suitable for the species in question but currently dominated by monocultures or grasslands. We propose this as the joint land sparing/sharing approach (SPASHA) and suggest it as a possible strategy to balance biodiversity conservation and sustainable vanilla production. Land sparing and land sharing have been projected as opposing strategies, where both social and biophysical factors determine which approach is most feasible or appropriate in a given

landscape (Fischer et al., 2011, 2014b; Hulme, 2011; Tscharntke et al., 2012; Chandler et al., 2013; Edwards et al., 2014; Gilroy et al., 2014). In the case of vanilla, we see a clear added value in the combination of both strategies, allowing the protection of natural forests holding *Vanilla* CWRs while surrounding, degraded lands can be used for the conversion into sustainable vanilla cultivation systems. For example, current grasslands or monocultures laying between forest fragments could be (partly) reforested for the creation of biological corridors where native trees species, who preferably provide economically interesting products, are planted and vanilla is cultivated as the cash crop. These agroforestry systems provide multiple ecosystem services and environmental benefits related to carbon sequestration, biodiversity conservation, soil enrichment and air and water quality (Jose, 2009). Furthermore, this approach allows local stakeholders in Costa Rica to get support from the Payments for Ecosystem Services (PES) program, because they (a) reforest degraded areas for the cultivation of, in this case, vanilla within agroforestry systems and (b) conserve surrounding forests where natural vanilla populations occur (Sánchez-Azofeifa et al*.,* 2007; Pagiola, 2008; Farley & Costanza, 2010). The latter is especially important because our SPASHA approach suggests, amongst others, the exchange of important natural interactions between forest and farmland.

An example of such an interaction is animal pollination, a crucial activity for the reproduction of plants that depend on specific animals for their pollination, which is also the case for *Vanilla* species. Since the invention of the hand-pollination technique by Edmond Albius in 1841, vanilla flowers in Madagascar's plantations have been pollinated manually and this successful technique is now applied worldwide (Havkin-Frenkel & Belanger 2018; Cameron, 2011). So far, there is evidence from own field observations and a few previous studies (Lubinsky et al., 2006; Pansarin & Pansarin, 2014, Pansarin & Miranda, 2016) that both the commercial species *Vanilla planifolia* and wild species within the section *Xanata* are naturally pollinated by bees of the Euglossini tribe (Apidae). Very little is known about the pollinator species or their pollination success, but observations demonstrate that the bee species responsible for pollination vary upon *Vanilla* species, probably due to differences in flower size, posing a reproductive barrier between species. In general, it is known that bees provide important ecosystem services to agriculture, including pest control and crop pollination, and agricultural landscapes can in turn provide habitats for several bee species (Bianchi et al., 2006; Klein, 2009; Kennedy et al., 2013; Martins et al., 2015). This important group of animals is threatened by the loss of natural and semi-natural habitats, extensive monoculture plantations, and increased pesticide and herbicide use (Freitas et al*.*, 2009). In the case of vanilla, we are convinced that our proposed SPASHA concept can protect

both *Vanilla* CWRs, and CWR-pollinator as well as other ecological interactions. However, further research on natural pollination is highly needed to evaluate the success of the SPASHA approach.

CWRs have long been recognized as priority species for conservation because their extinction threatens the genetic base of many of the world's crops (Heywood et al., 2007; Castañeda-Álvarez et al., 2016). Their protection started with *ex situ* conservation programs, which were not as successful as expected, especially for species that are characterized by small and dispersed, genetically distinct populations with low seed viability and complex interspecific relationships within their natural habitat, which is the case for *Vanilla* species (Alomia et al., 2017; Azofeifa-Bolaños et al., 2018). High maintenance costs of clonal collections and problems regarding the regeneration of stored material and seed recalcitrance pose additional challenges for the *ex situ* conservation approach (Pritchard et al., 2012; Braverman, 2014). Therefore, interest in *in situ* conservation (Harlan, 1992) has increased over the years and is now considered a key element to conserve CWRs. Joint land sparing/sharing is an example of *in situ* conservation (i.e. the conservation of forests with natural vanilla populations) and *ex situ* cultivation (the development of areas where promising *Vanilla* CWRs will be cultivated).

The starting point of CWR conservation are large-scale inventories (Gadgil, 1993). In 1985, Bioversity International established a list of genera for priority surveys and *in situ* conservation (IBPGR), stimulating country's interest and action towards CWR inventories. Hodgkin (2001) stressed the priority of endangered species given the potential negative impacts of climate change and habitat fragmentation on unstable and isolated populations (Wilcove & Chen, 1998, Brigham et al., 2002; Pitman et al., 2002). Many species within the orchid family are currently facing extinction risks. Despite substantial conservation emphasis on rare orchids, populations continue to decline (Gale et al., 2018). Almost all the species within the genus *Vanilla* that have been assessed in the IUCN Red List are listed as endangered (Herrera-Cabrera et al., 2017; Wegier et al., 2017). Their natural populations are threatened by deforestation and land degradation as well as uncontrolled harvest from wild populations (Schlüter et al., 2008; Flanagan & Mosquera-Espinosa, 2016). Since vanilla is a high-value crop facing several production problems, and given that its CWRs hold beneficial features for crop improvement, we suggest the listing of its CWRs and the start of large-scale inventories along its distribution range as high priority actions. Especially because these results can be applied into management plans on a national and broader scale.

2.5.3 Implementation in land use policy

The present study emphasizes the proposed management interventions within the biological corridors as part of the National Biocorridor Program (PNCB) and surrounding forest fragments. The PNCB, a network of connecting structures between state conservation areas, was created in 2006 and reformed in 2017 as a strategy of biodiversity conservation. The goal of this program is to promote the conservation and sustainable use of the biodiversity in Costa Rica, from the perspective of a functional and structural ecosystem connectivity (SINAC, 2018). Up to present, a strategic plan of the protection and management of these corridors in collaboration with local stakeholders (Plan Estratégico 2018-2025, as part of PNCB) is formed but no action has been taken yet. There is a plan for the establishment of incentive systems and alternative income-generating opportunities within these corridors, where the involvement of local parties is key. As one of the first in contributing to this plan, we propose the implementation of the SPASHA approach within these corridors and their surroundings through the creation of diverse and productive landscapes with integration of *Vanilla* CWRs as a high-value certified crop on degraded lands, while protecting the surrounding natural forests. Besides the corridor Osa, we also consider the suitable areas within the Golfo Dulce forest reserve and its surroundings as areas of importance. Although defined as a forest reserve, personal annotations and observations from MINAE-SINAC indicate that this area is still exposed to deforestation for land conversions and illegal logging. With our approach, we could provide sustainable land use practices within these areas to prevent this from happening. It also contributes to the goals of the PNCB, i.e. to give these areas an environmental and economic value, thereby offering alternative income generation for local communities.

2.6 CONCLUSIONS

Costa Rica is recognized for its positive attitude towards biodiversity conservation strategies and natural resource management in general, with clean energy production, the creation of 11 conservation areas, an extensive national park system and a national biological corridor network as prime examples. However, unprotected lands are still being converted into rather unsustainable land use practices, with the main agricultural practices focusing on monocultures of oil palm, pineapple, banana and teak or grasslands for cattle grazing. Moreover, it is the leading country regarding the use of biocides per unit area (Faostat, 2021). Therefore, Costa Rica serves as an interesting country to apply the joint land sparing / land sharing (SPASHA) strategy. Given the growing awareness of the economic potential of *Vanilla* species, they should receive priority

action for conservation and sustainable use, recently pointed out by amongst others Flanagan and Mosquera-Espinosa (2016) in Colombia and Herrera-Cabrera et al. (2018) in Mexico. We propose the incorporation of the SPASHA strategy within land management plans in order to reduce the increasing pressure of unsustainable extraction from natural populations and to promote sustainable cultivation practices. For this study, we focused on the corridor Osa because of the availability of a recent land use map of our study region ACOSA. Future research should involve the creation of recent, high quality land use maps of the whole country, to implement our findings regarding land management plans within the biological corridors and their surroundings at a national level. We also suggest to continue collecting records on *Vanilla* CWRs through forest inventories and collaboration between countries within the native ranges of aromatic *Vanilla* species. This to apply the SPASHA approach on a broader scale and especially to better understand the distribution, taxonomy, biology, ecology and potential use of the different *Vanilla* CWRs along the Neotropics. By using modern technologies such as iNaturalist, local communities can get involved in the gathering of georeferenced data as well as in the collection of data on flowering, fruiting and specific habitat preferences. Furthermore, we did not model the potential niche shifts of *Vanilla* species under climate change, but suggest this should be explored in a further study. The latter could additionally include data on the distribution of associated pollinators, seed dispersers, and soil and leaf litter microbial communities.


3

Chapter 3

Trick or Treat? Pollinator attraction in *Vanilla pompona* (Orchidaceae)

Adapted from:

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Photo: Flower of the *Vanilla* crop wild relative *Vanilla pompona* visited by its pollinator, an orchid bee belonging to the genus *Eulaema* (*Eulaema cingulata*). © Charlotte Watteyn

3.1 SUMMARY

Natural pollination of species belonging to the pantropical orchid genus *Vanilla* remains poorly understood. Based on sporadic records, euglossine bees have been observed visiting flowers of Neotropical *Vanilla* species. Our research aimed at better understanding the pollinator attraction mechanism of the Neotropical species *Vanilla pompona*, a crop wild relative with valuable traits for vanilla crop improvement programs. Using video footage, we identified floral visitors and examined their behavior. The flowers of *V. pompona* attracted *Eulaema cingulata* males, which distinctively displayed two behaviors: floral scent collection and nectar search; with the latter leading to pollen removal. Morphological measurements of floral and visitor traits showed that other *Eulaema* species may also act as potential pollinators. Additionally, we recorded natural fruit set in three populations and over a period of two years, tested for nectar presence and analyzed floral fragrances through gas chromatography - mass spectrometry. We observed a low natural fruit set (2.42%) and did not detect nectar. Twenty floral volatile compounds were identified, with the dominant compound trans-carvone oxide previously found to attract *Eulaema cingulata* males. We hypothesize a dual attraction of *Eulaema cingulata* males to *V. pompona* flowers, based on floral fragrance reward as the primary long-distance attraction, and food deception for successful pollen removal. Further research confirming this hypothesis is recommended to develop appropriate conservation policies for this and other *Vanilla* crop wild relatives, as they are the primary reserves of this crop's genetic variation.

3.2 INTRODUCTION

Plant-pollinator interactions are typically mutualistic, with a reward offered by the plant in exchange for a pollination service (Wright & Schiestl, 2009). Within the orchid family (Orchidaceae), approximately two-thirds of all species reward their pollinators with nectar, floral fragrances, oils or food hairs (van der Pijl & Dodson, 1966; Nilsson, 1992; Williams, 1982; Shrestha et al., 2020), while the remaining are believed to be deceptive (Dafni, 1984; Ackerman, 1986). Among the floral fragrance-rewarding species, odor-seeking male orchid bees (Apidae: Euglossini) have been reported to be responsible for the pollination of hundreds of Neotropical orchid species (Dodson et al., 1969; Williams & Whitten, 1983). These males gather volatile compounds from floral or other sources to be used as courtship attractants, a phenomenon known as the "Perfume Flower Pollination Syndrome" (PFPS) (Dodson, 1975; Kimsey, 1984; Schemske & Lande, 1984; Eltz et al., 2003, 2005; Cappellari Rabeling & Harter-Marques, 2010).

The pantropical orchid genus *Vanilla* Plum. ex Mill. (1754) consists of 118 species (Karremans et al., 2020). Only those native to the Neotropics and belonging to the section *Xanata* produce aromatic pods or beans, such as *Vanilla planifolia* Andrews and its crop wild relatives (CWRs). Commercial vanilla plants are almost exclusively hand-pollinated, leading to satisfactory bean yields (Havkin-Frenkel & Belanger, 2018; Hu et al., 2019). Vector-mediated natural pollination, however, generally results in cross-pollination and increases genetic variability. The latter augments survival probabilities in changing environments and contributes to the development of potentially interesting crop traits related to, amongst others, pest resistance and bean quality. Studies on natural vanilla pollination are scarce but crucial to safeguard the long-term survival of this crop and its CWRs, which are the primary reserve of a crop's genetic variation and a prerequisite for crop improvement programs (Castañeda-Álvarez et al., 2016; Flanagan & Mosquera-Espinosa, 2016). The intrageneric diversity in floral morphology suggests the evolution of different pollination mechanisms (Cameron & Soto Arenas, 2003; Soto Arenas & Dressler, 2010). Even though spontaneous autogamy has been reported (Bateman et al., 2004; Van Dam et al., 2010; Pansarin, 2016), most *Vanilla* species are suspected to depend on animal vectors for pollination (Pansarin et al., 2014; Gigant et al., 2016; Chaipanich et al., 2020). Members of the Euglossini tribe are suggested as pollinators of the Neotropical species (Ackerman, 1983a; Lubinsky et al., 2006; Householder et al., 2010; Soto Arenas & Dressler, 2010; Pansarin & Pansarin, 2014; Anjos et al., 2017), but the specific mechanisms are still poorly understood. Our study aimed at better understanding the pollinator attraction mechanism and reproductive biology of the Neotropical species *Vanilla pompona* Schiede (*Vanilla* sect. *Xanata*), by (a) identifying its

floral visitors and comparing their behaviors, (b) confirming its pollinator species and quantifying natural fruit set, (c) identifying floral rewards, and (d) predicting an optimal morphological fit between flower and pollinator to determine other potential pollinator species.

3.3 MATERIALS AND METHODS

3.3.1 Study area and species

The study took place in Costa Rica and Peru, in respectively the Osa Peninsula and the Madre de Dios region (Fig. 3.1).

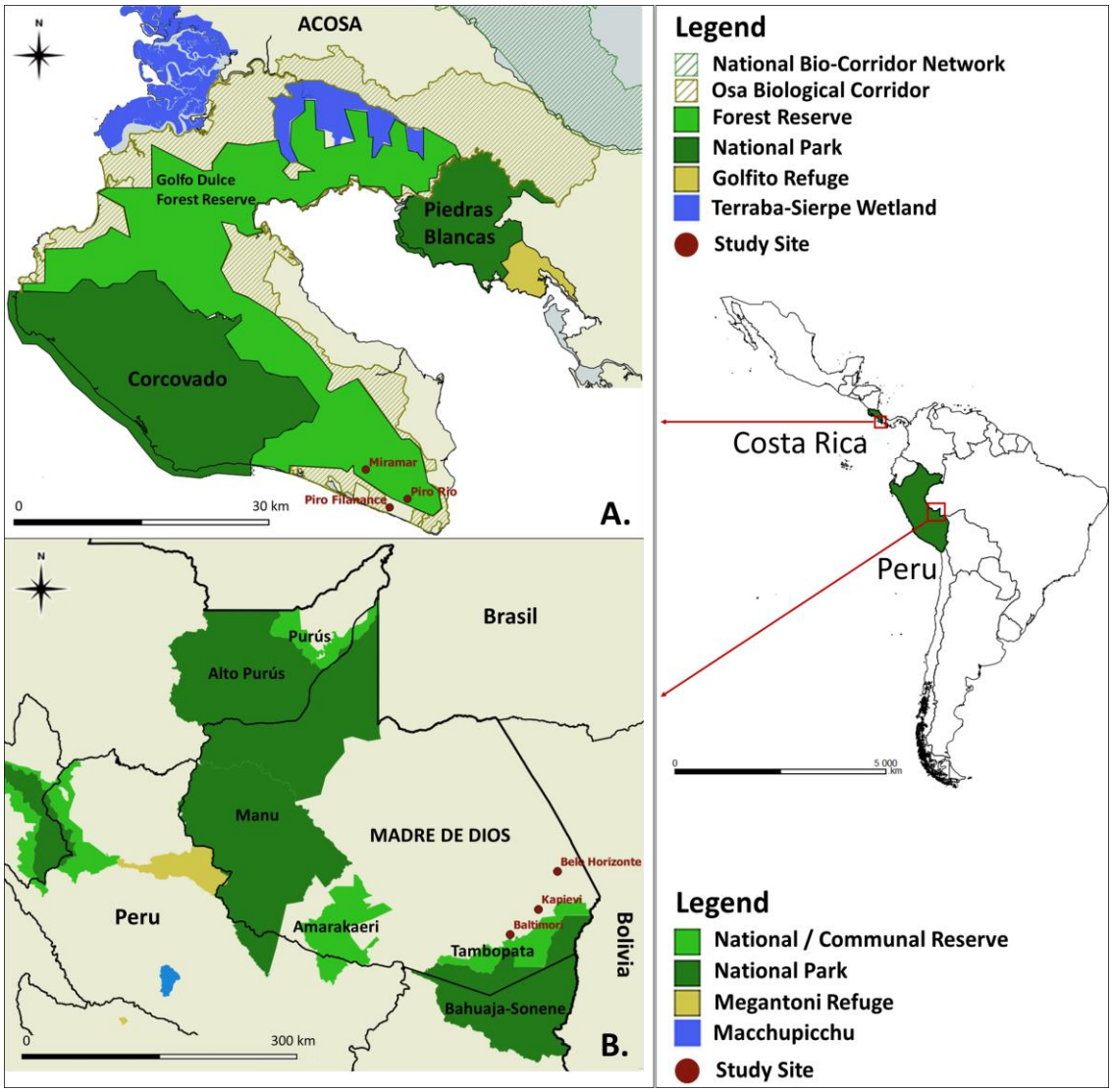

Fig. 3.1. *Map showing the study sites in (A) the Osa Peninsula, Costa Rica (Miramar and Piro), and (B) the Madre de Dios region, Peru (Baltimore, Kapievi, Belo Horizonte). Both areas are biodiversity hotspots that include protected areas such as national parks, communal or national forest reserves, and biological corridors (Kohlmann et al., 2010).*

The habitat of the study sites is characterized by secondary tropical rainforests and floodplain wetlands surrounded by humid tropical forest in, respectively, Costa Rica and Peru. The Osa Peninsula has an average annual precipitation ranging from 2,500 to 6,000 mm, and an average annual temperature of 25°C. The annual average rainfall in the Madre de Dios region ranges from 2,000 to 3,500 mm and average temperatures from 21°C to 26°C (Atrium Biodiversity Information System, 2011; Holdridge, 1967; Kappelle, 2003). The Osa Peninsula hosts six *Vanilla* species: *Vanilla inodora* Schiede, *V. hartii* Rolfe, *V. helleri* A.D. Hawkes, *V. odorata* C. Presl, *V. pompona*, and *V. trigonocarpa* Hoehne (Karremans et al., 2020; Watteyn et al., 2020). The Madre de Dios regions also hosts six species: *Vanilla pompona*, *V. marowynensis* Pulle, *V. karen-christianae* Karremans & P.Lehm., *V. bicolor* Lindl, *V. palmarum* Salzm. ex Lindl., and *V. guianensis* Splitg (Peru) (Householder et al., 2010; Janovec et al., 2013).

Vanilla pompona, listed by the IUCN as Endangered (Herrera-Cabrera, 2020), has a distribution from Mexico to Brazil and Peru (Soto Arenas & Dressler, 2010). Flowers consist of three sepals, two lateral petals, and one large trumpet-shaped petal known as the lip or labellum. The latter is partially fused to the column, which bears a single ventral stigma, separated by the rostellum from the anther holding two pollinia-like pollen masses, that are deposited as smears. Each flower lasts for one day and starts to wither after midday.

3.3.2 Floral visitors and their behavior

Floral visitors were observed in the Costa Rican and Peruvian study sites (hereafter *V. pompona* populations) during the flowering period of January-February 2019 (120 h of observation) and September 2018 (64 h of observation), respectively. We checked for newly-opened flowers and observed them from 0600 h to 1400 h, corresponding to the time of anthesis until the flowers start to wither. Cameras (CANON SX60 HS, EOS M) were located at a minimum distance of three meters from the flower to avoid disturbance. Videos were recorded upon visitor approaches (Costa Rica) or with tele macro mode activated (Peru). We screened the video footage to identify floral visitors, record their visiting time, and examine their behavioral patterns.

Subsequently, we assigned them to five behavioral categories, following the behavioral hierarchy of bee attraction: (a) approaching, (b) landing on tepals or labellum, (c) landing on labellum, (d) entering labellum, and (e) successfully removing pollen. To model the effect of floral visitor genus and time of the day on this ordinal bee behavior classification, we used a series of mixed logistic regression models, and sequentially compared each pair of consecutive behavior categories. For example, the first model fits the chance of landing, given a bee approached the flower, as a

function of bee genus and time of the day. We accounted for the correlation structure in our data by including random intercepts for flower individual nested within location (Zuur et al., 2009), and the assumption of linearity between the log odds and continuous predictor variable was checked. Due to non-convergence problems with glmmTMB, we applied a Bayesian multilevel model. The models were fit using the R package brms (Bürkner, 2017) with 2 chains, 10,000 iterations and a warm-up of 1000 runs. Using normal priors for intercept and betas, models converged with Rhat values close to 1 (Gelman and Rubin's diagnostic). Trace plots were used to assess mixing and convergence of the two chains. Parameter estimates of models including only one or both random effects were compared and models were validated via posterior predictive checks, such as the widely applicable information criterion (WAIC; Watanabe, 2010) and the leave-one-out cross-validation (LOO; Gelfand et al., 1992; Vehtari et al., 2017), using the bayesplot R package (Gabry, 2017).

3.3.3 Pollinator identification and pollination success

The floral visitors that removed pollen were identified at species level and determined by sex. Additionally, bees carrying pollen were caught during the flowering period in Costa Rica and Peru, using baits shown to be attractive compounds: eugenol, 1,8-cineole, and methyl salicylate (Dressler, 1981; Ackerman, 1989; Roubik & Hanson, 2004). The identity of the pollen was confirmed by morphological comparison with *V. pompona* pollen using high microscope magnification. Bees were identified using the key in Roubik and Hanson (2004). To test for autogamy, ten inflorescences from two Peruvian populations were bagged the day before anthesis with an insect proof nylon bag using a ground-based tool (Scaccabarozzi et al., 2020).

Fruit set within the Costa Rican populations was quantified during two flowering periods (2019 and 2020). We counted the number of inflorescences per population, flowers per inflorescence, and developed fruits per inflorescence. The calculated fruit set was then compared among the three populations using the non-parametric Kruskal-Wallis test and Dunn's post-hoc tests with multiple comparison correction (Benjamini-Hochberg adjustment to control the False Discovery Rate) and the two flowering periods using the non-parametric Mann-Whitney test.

3.3.4 Floral rewards

Nectar

To determine if pollination is based on a nectar reward, we bagged flowers the day before anthesis (Corbet, 2003) and visually inspected 20 flowers from the Costa Rican populations for secretory

glands under a stereoscopic microscope. Attempts to collect nectar using microcapillary tubes (2 µL) were undertaken in 10 of these flowers. Since no secreted fluids were observed or collected, we applied the rinsing method for small nectar volumes (Morrant et al., 2009; Power et al., 2017) in eight additional flowers. The rinsed water was analyzed using High-Performance Liquid Chromatography (HPLC) in an Agilent 1260 Infinity system (Santa Clara, USA) with refractive index detector (G1362A). Three successive rinses (0.5 mL each) of de-ionized UHPLC gradient water were expelled over the labellum. No floral tissue was damaged or removed from the plant to avoid possible leakage of other tissue fluids. Since direct injection of a sample did not detect masses of nectar sugars (sucrose, glucose and fructose), 1 mL of a sample was lyophilized using 1-propanol HPLC grade and evaporated with nitrogen. This was then diluted in 30 µL mobile phase (ultrapure water type 1, Milli-Q®, Millipore Corporation, Burlington, USA) to concentrate the sugars 33 times more to quantify them. Sugar separation was achieved by injecting 9 to 15 µL of this solution into a Hi-Plex Ca2+ column (300 × 7.7 mm, 8 µm, Agilent, Santa Clara, USA) at 45 °C, in isocratic mode with a flow of 0.5 mL/min. A 25 min chromatographic run achieved separation and the limits of detection (LOD) was determined by the signal-to-noise ratio (S/N ratio = 3).

Floral fragrances

Floral fragrances of 12 flowers were collected by static headspace sampling (Tholl et al., 2006) during the 2020 flowering period in two Costa Rican populations. The sampling was conducted between 7:00 am and 11:00 am, corresponding to the activity peak of potential pollinators (Dodson et al., 1969; Ackerman, 1983b; Armbruster et al., 2000; Gostinski et al., 2016), thus expected floral scent productivity peaks (Whitten, 1985; Hills & Williams, 1990). The single-use scent traps were constructed using clear glass tubing (2.4 mm ID, 3.5 cm length). They were plugged at both ends with glass wool, filled with 20 mg of bulk carbon and 20 mg of Tenax GC (Williams & Whitten, 1983; Whitten, 1985; Raguso & Pellmyr, 1998), and conditioned by passing 5 mL of hexane. During each sampling, one inflorescence was bagged with a plastic scentless oven bag (Reynolds Kitchens) closed at the top for 30 min. The scent traps were then connected with the tenax-side to the bag and the carbon-side to a battery-operated vacuum pump via Tygon tubing (ID 3.3 mm). For three hours, air was continuously extracted from the bag through the scent trap and the flow rate was adjusted to 200 mL/min using a 9 V battery. The scent traps were eluted with 200 µL of hexane into conical inserts held in 2 mL auto-sampler vials (Agilent Technologies), stored at -20°C until analysis. Control samples were collected simultaneously from bags filled with ambient air. The scent traps were analyzed by gas chromatography-mass

spectrometry (GC-MS) using an Agilent 7890B GC fitted with a 30 m x 0.25 mm x 0.25 µm HP-5 Ultra Inert column coupled to an Agilent 5977A mass spectrometer (Agilent Technologies). We used the same methodology for data analysis and processing, and compound identification and attractiveness as described in Hetherington-Rauth & Ramirez (2016). Presumed contaminant compounds were removed when present in both control and sample to obtain the final set of floral volatile compounds. We compiled information on the attractiveness of each of the final compounds from the literature, including field behavioral assays (Dodson et al., 1969; Williams & Dodson, 1972; Williams & Whitten, 1983; Ackerman, 1989; Hetherington-Rauth & Ramirez, 2016). The compound peak areas of the final set of floral volatiles were converted to relative frequencies and averaged per compound to reveal potential patterns within the floral bouquet.

3.3.5 Flower and floral visitor morphology

We measured and compared pollinator dimensions with floral traits to assess the pollinator morphological-fit to flower structure. Bee traps were located in the vicinity of the Peruvian and Costa Rican populations. The same methodology was used as described in Section 3.3.3. The bees' total length, mesosoma height and diameter, and extended tongue length were measured using a caliper. Next, 32 *V. pompona* flowers were measured across the Costa Rican populations. The measured traits were: height (H1), width (W) and length (L1) of the labellar tube, height of the labellar tube below the anther (H2), thickness of the column at height of the anther (T_Co), height of the labellar callus (T_Ca), height of the labellar keels (T_La), and pedicel length (L2) (Fig. 3.2).

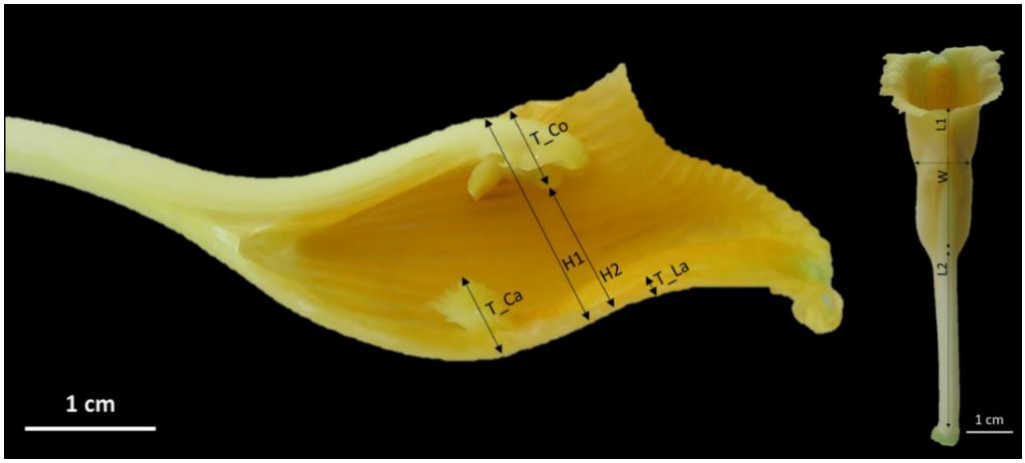

Fig. 3.2. *Measured V. pompona floral traits: height (H1), width (W) and length (L1) of the labellar tube, height of the labellar tube below the anther (H2), thickness of the column at the height of the anther (T_Co), height of the labellar callus (T_Ca), height of the labellar keels (T_La), and length of the pedicel (L2). (vouchers at JBL)*

Further derived variables were estimated to assess the size of the labellar tube: “An”, labellar tube entrance measured at the apex of the anther (H2 - T_La) and “Cn”, labellar tube measured at the height of the callus, below the rostellar flap (H2 - T_Ca).

3.4 RESULTS

3.4.1 Floral visitors and their behavior

Of the 123 recorded floral visitors, 82.1% were Euglossini, belonging to the genera *Eulaema* (n = 73) and *Euglossa* (n = 28), and 17.9% were Trigonini of the genus *Trigona* (n = 22). The behavioral comparison analysis showed that most bees approaching the flowers subsequently landed on them (Table A2.1). Closer towards the end of the morning, the chance that a bee lands on a flower decreases significantly (Fig. A2.1). Approaching *Euglossa* or *Eulaema* are predicted to land more on the tepals than on the labellum, where they show a probing behavior of brushing the flower structure with their foretarsi (Table 3.1). The chance that an approaching *Trigona* lands on the labellum edge or enters the labellar tube is higher compared to *Euglossa* or *Eulaema*, and all *Trigona* display a probing behavior of touching the flower surface with their proboscis or antennae. *Eulaema* or *Euglossa* that enter the labellum, visit the flower for less than five seconds and remain inside for about two seconds. As they exit, an extended tongue was observed. These rapid visits suggest unsuccessful attempts of nectar foraging. Despite the observation of three different bee genera in both Costa Rica and Peru, only individuals belonging to the genus *Eulaema* (n = 5) successfully removed pollen when entering the labellum (Fig. A2.2). Overall, landing bees display two main reward-approach behaviors on different flower parts: probing (Video 3.1) or attempts for nectar search (Video 3.2).

Table 3.1. *Percentage of landing bees per genus displaying a defined reward approach type on different parts of Vanilla pompona flowers (T = tepals; LE = labellum edge; LT = labellum tube), whereby “probing” refers to touching the flower surface with the proboscis and antennae and / or brushing with foretarsi, and “nectar search” refers to entering straight into the labellum chamber, and showing an extended tongue when entering or departing the flower.*

Reward approach	***Eulaema*** **(N[1] = 61)**			***Euglossa*** **(N[1] = 23)**			***Trigona*** **(N[1] = 22)**			**Visit time (s) (mean ± SD[2])**
	T	LE	LT	T	LE	LT	T	LE	LT	
Probing	80.3	1.6	-	95.6	-	-	4.5	54.6	40.9	373.5 ± 926.6
Nectar search	-	-	18.1	-	-	4.4	-	-	-	4.7 ± 2.5

[1]N = total number of landing bees per genus.
[2]SD = standard deviation.

3.4.2 Pollinator identification and pollination success

The five bees observed removing the pollen of *V. pompona* flowers were identified as male *Eulaema cingulata*. Furthermore, using chemical baits, we caught five male *Eulaema cingulata* that carried *V. pompona* pollen masses attached to their scutellum (Fig. 3.3). Fruit set at inflorescence level was significantly different among the populations ($H(2) = 7.72$, $P = 0.02$), with Piro-Filanance having a higher fruit set compared to Miramar ($P = 0.01$) and Piro-Rio ($P = 0.02$) (Table 3.2). No difference was found between the two years ($H(1) = 0.42$, $P = 0.52$). The autogamy test resulted in zero fruit development.

Table 3.2. *Mean fruit set (%) of Vanilla pompona populations at the three study sites in Costa Rica, measured during the flowering period of 2019 and 2020.*

	Miramar (mean ± SD[1])	**Piro-Filanance (mean ± SD[1])**	**Piro-Rio (mean ± SD[1])**
Year 2019	0.77 ± 3.92	5.35 ± 11.10	1.39 ± 5.07
Year 2020	1.93 ± 5.81	4.30 ± 8.68	1.04 ± 3.87

[1]SD = standard deviation.



Fig. 3.3. *Caught male Eulaema cingulata with attached pollen of V. pompona and extended tongue (A), and attached V. pompona pollen in close-up (B). (vouchers at JBL)*

3.4.3 Floral rewards

No nectaries were observed after visually inspecting 20 *V. pompona* flowers and no nectar could be collected via microcapillary tubes. The rinsing method followed by HPLC analysis detected total sugars (glucose, fructose, sucrose) in trace (< 0.03 mg) amounts (four samples) or non-quantifiable amounts (five samples) (Fig. A2.3). We detected a total of 20 floral fragrance

compounds emitted by *V. pompona* flowers (Table 3.3). One of them could not be identified. The most representative compounds of the floral bouquet were trans-carvone oxide (35.70 ± 14.85%), limonene (17.45 ± 6.00%), limonene oxide, trans (16.72 ± 6.87%) and limonene oxide, cis (8.97 ± 6.80%), followed by terpinolene (6.94 ± 3.60%), carvone (4.42 ± 1.50%), myrcene (3.34 ± 2.36%), 1-R-alpha-pinene (2.61 ± 2.30%) and (-)-beta-pinene (1.33 ± 0.93%) (Fig. A2.4). Other compounds were present in low amounts (mean relative peak area < 1%).

Table 3.3. *Identified floral volatile compounds, Kovats retention indices, retention times (min), and mean relative peak areas (%) calculated from the total peak area in the GC-MC chromatogram from V. pompona flowers (N = 12).*

Compound	Kovats retention index[1]	Retention time[1] (min)	Relative peak area (%)[2] (mean ± SD[3])
alpha-pinene oxide	1077.59	7.69	0.04 ± 0.04
1-R-alpha-pinene	934.44	5.18	2.61 ± 2.30
(-)-beta-pinene	977.62	5.95	1.33 ± 0.93
cis-carveol	1222.48	10.03	0.15 ± 0.07
carvone	1248.58	10.42	4.42 ± 1.50
trans-dihydrocarvone	1207.43	9.81	0.32 ± 0.10
trans-carvone oxide	1277.30	10.84	35.70 ± 14.85
cis-carvone oxide	1283.64	10.94	0.70 ± 0.32
limonene	1031.50	6.89	17.45 ± 6.00
limonene oxide	1116.07	8.35	0.22 ± 0.09
limonene oxide, cis	1140.32	8.74	8.97 ± 6.80
limonene oxide, trans	1147.06	8.85	16.72 ± 6.87
S-(-)-limonene	1327.05	11.55	0.35 ± 0.16
linalool	1097.99	8.06	0.05 ± 0.11
myrcene	990.73	6.35	3.34 ± 2.36
cis-beta-ocimene	1048.99	7.20	0.35 ± 0.13
cis-beta-terpineol	1068.62	7.54	0.03 ± 0.03
terpinolene	1093.05	7.97	6.94 ± 3.60
L-4-terpineol	1190.86	9.55	0.15 ± 0.07
unknown-1	1199.53	9.69	0.16 ± 0.07

[1]Retention time (minutes) and Kovats retention indices for each floral compound; [2]Mean relative peak area and standard deviation of floral compounds, calculated by dividing the corresponding floral compound peak area by the total peak area of all floral compounds per sample, and averaging the samples. The sum of the peak areas of all identified compounds was 1.29 x 10^9 mV/sec. [3]SD = standard deviation.

3.4.4 Flower and floral visitor morphology

A total of 83 *Eulaema* (3 species), 690 *Euglossa* (20 species), and 32 *V. pompona* flowers were measured. The trait “An” (8.40 ± 0.35 mm) and “Cn” (6.95 ± 0.36 mm) showed the best fit with the height of the pollinator species *Eulaema cingulata* (7.73 ± 0.31 mm) (Fig. 3.4). In fact, *Eulaema cingulata* fits exactly between these two traits. The *Euglossa* species (3.95 ± 0.58 mm) were too small to fall into the An-Cn gap, whereas the other *Eulaema* species, *Eulaema bombiformis* (9.39 ± 0.39 mm) and *Eulaema meriana* (8.98 ± 0.44 mm), were too large.

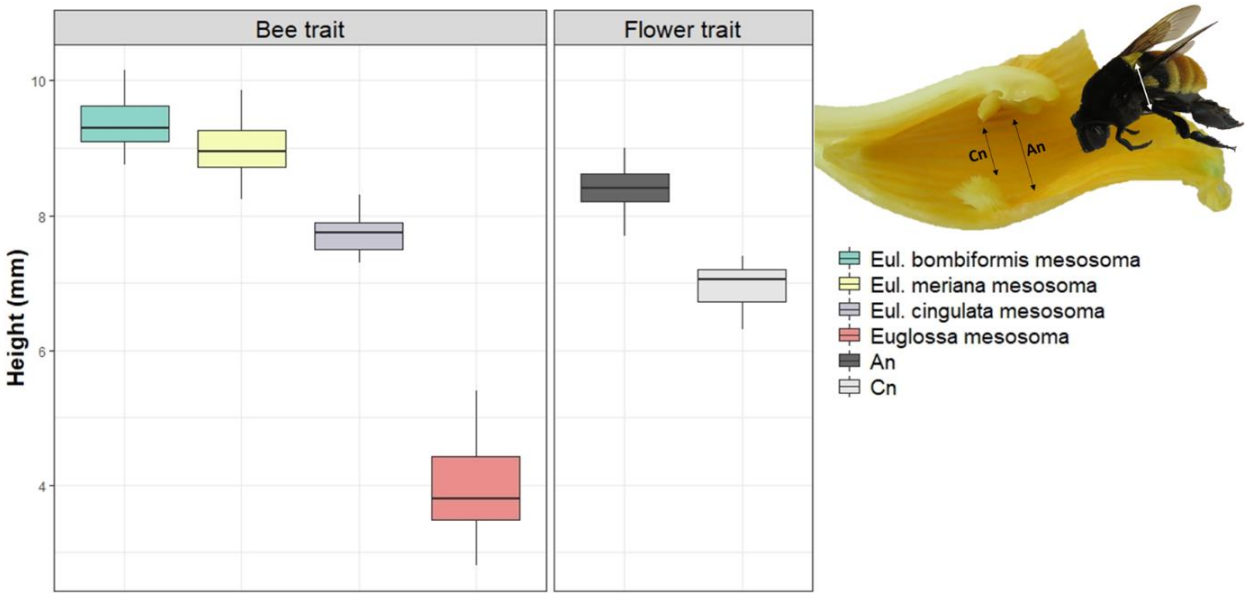

Fig. 3.4. *Comparison of the mesosoma heights (mm) of the bee species Eulaema bombiformis, Eulaema meriana, Eulaema cingulata and Euglossa spp. with the flower morphological traits “An” (mm) and “Cn” (mm) of V. pompona.*

3.5 DISCUSSION

Vanilla pompona flowers are visited by bees of the genera *Eulaema*, *Euglossa* and *Trigona*. As confirmed by both the autogamy test and video footage, sexual reproduction is vector-mediated. Only male *Eulaema cingulata* were observed removing pollen and caught with *V. pompona* pollen masses. They are therefore suspected to act as effective pollinators in both Costa Rica and Peru. Yet, it is not possible to exclude females nor other species as potential pollinators.

Bee behavioral patterns were assessed and compared. Towards the end of the morning, a decrease in number of landing bees suggests that flowers become less attractive due to senescence close to midday or after previous floral visits, as reported earlier for several plant species (van Doorn, 1997). *Trigona* landed almost exclusively on the labellum and exhibited a

probing behavior indicative of foraging (Armbruster, 1984; Slaa et al., 1998; Nieh, 2004; Lichtenberg et al., 2010; McCabe & Farina, 2010; Grajales-Conesa et al., 2012). *Euglossa* and *Eulaema* were observed more frequently on the tepals, repeatedly brushing with their foretarsi, then hovering around while transferring compounds to their hind tibia, indicating fragrance collection (Dressler, 1968; Whitten et al., 1989; Eltz et al., 2003). Some *Euglossa* and *Eulaema* did not collect fragrances but entered directly into the labellum. These visits were brief, after which they quickly exited the flower showing an extended tongue, suggesting nectar search. Only *Eulaema cingulata* successfully removed the pollen after searching for nectar in the labellum.

3.5.1 The dual pollinator attraction hypothesis

The floral volatile compound composition was quantified for the first time in *V. pompona*. Trans-carvone oxide, limonene and limonene oxide together represented 78.84% of the floral perfume. Previous field bioassays have demonstrated that the compound trans-carvone oxide attracts *Eulaema cingulata* and other *Eulaema* species, that collect and store it in their hind legs (Whitten et al., 1986, Etlz et al., 2006; Milet-Pinheiro & Gerlach, 2017). Flowers of *Catasetum* species, known to be pollinated through PFPS by different *Eulaema* species, also contain trans-carvone oxide (Whitten et al., 1986; Gerlach & Schill, 1991; Milet-Pinheiro & Gerlach; 2017). Hence, this compound most likely evolved in perfume-rewarding plant species as an attractant for *Eulaema* pollinator species (Whitten et al., 1986; Brandt et al., 2019). The specific role of the other identified compounds and their interaction with the *Eulaema* attractant trans-carvone oxide could be explored further since studies have demonstrated that appropriate compound blends filter out certain bee pollinator species (Williams & Dodson, 1972; Whitten et al., 1986).

For pollination to occur, bees need to actively crawl into the labellum. Curiously, none of the observed male *Eulaema cingulata* exhibiting fragrance collection behavior on the tepals subsequently moved into the labellum. We distinctly observed male *Eulaema cingulata* entering the labellum and exiting the flowers with extended tongues, indicative of nectar search. The identification of sugar traces suggests that *V. pompona* flowers deceive their pollinators, which is conform with our observations of scarce and speedy visits in the labellum and a low natural fruit set (2.42%) (Scopece et al., 2010; Scaccabarozzi et al., 2020). To tease apart whether the deception is based on resembling a general flower image or a specific model species (Johnson and Schiestl, 2016; Caballero-Villalobos et al., 2017), advertising signals shared by *V. pompona* and co-occurring *Eulaema cingulata*-pollinated species could be explored. Sugar traces most likely encourage the bees to crawl inside the labellum, but there exists quite some debate whether

to decide if this is a food deceptive or semi-rewarding strategy. In case of a semi-rewarding strategy, however, we would expect revisits since traces do not satisfy the bees' high energy demands. In accordance with Shrestha et al. (2020), we suggest that sugar traces are deceitful, but the nature of the deceit is more complex compared to complete nectar absence and definitely requires a more in-depth comprehension.

Combining our behavioral and phytochemical data, we hypothesize a dual pollinator attraction in *V. pompona* flowers, employing floral fragrance rewards as initial attraction and food deception to induce pollen removal and deposition. The volatile compounds, especially trans-carvone oxide, possibly provoke the primary long-distance attraction of male *Eulaema cingulata* to a flowering population. *V. pompona* flowers only last for one day and start to wither after midday. This restricted time lapse may have led to the development of floral fragrance rewards to boost pollinator attraction. Yet, the attempt for nectar search is essential for pollen removal and seems to occur via food deception as the short-distance attraction, taking place when these male bees, now present in the surroundings of the flowering *V. pompona*, are foraging for food resources. Studies involving flower manipulation experiments (i.e. removing fragrant compounds) or bee tagging could confirm our proposed hypothesis and examine if this mechanism has been evolved across other Euglossini-pollinated *Vanilla* species.

4.5.2 Morphological goodness-of-fit for pollen removal by *Eulaema*

We assessed the "goodness-of-fit" between flower and bee to ensure pollen removal and showed that the body height of the pollinator *Eulaema cingulata* perfectly fits in between the flower traits "An" and "Cn". Given that vanilla flowers have a single aperture for entrance and exit, the callus serves as a positioning structure, bringing the bee close to the rostellum. This results in the latter being moved backwards, displacing the anther and releasing the pollen on the bee's scutellum. It explains why the height of the labellar tube at the callus is slightly smaller than the bee height and demonstrates the key role of the labellar tube and the callus, both highly conserved features in *Vanilla* sect. *Xanata*. Since the chemical baits did probably not attract all available *Eulaema* species and the floral and bee morphological traits show intraspecific variation, more bee species could fall within the "An-Cn gap". This is beneficial from a plant's perspective since dependence on a single pollinator species might hazard a plant's population survival, especially when subjected to environmental pressures (González-Varo et al., 2013; Traveset et al., 2017).

3.5 CONCLUSIONS

We hypothesize that *V. pompona* pollination by male *Eulaema cingulata* is based on floral fragrance attraction towards a flowering population and food deception to induce pollen removal and deposition. This proposed dual pollinator attraction mechanism can be explained by the need to (a) attract bees from long distances to flowering populations, and (b) lure bees into the labellum for successful pollination. Our study offers novel insights into plant-pollinator interactions within the *Vanilla* genus and could be corroborated by further research on the attraction of both sexes to *V. pompona* flowers, the presence of sugar traces as a semi-deceptive mechanism, the pollinator species diversity across *V. pompona*'s distribution within the Neotropics, and the effects of environmental changes on these interactions. Pollination studies are essential to define proper conservation policies, thereby preserving the natural habitat of both vanilla and its pollinators and assuring their long-term survival and agricultural application.


4

Chapter 4

Sweet as *Vanilla hartii*: Evidence for nectar rewards in *Vanilla* (Orchidaceae) flowers

Adapted from:

Watteyn, C., Scaccabarozzi, D., Muys, B., Reubens, B., Ackerman, J.D., Fernández Otárola M., Guizar Amador, M.F., Karremans, A. 2022. Sweet as *Vanilla hartii*: Evidence for nectar rewards in *Vanilla* (Orchidaceae) flowers. Submitted to Flora (under review).

Photo: Flower of the *Vanilla* crop wild relative *Vanilla hartii*. The white flower is prominent against the darker background of its natural habitat, the old-growth tropical rainforests. © Charlotte Watteyn

4.1 SUMMARY

Scant documentation exists on the pollination mechanisms of species belonging to the genus *Vanilla* (Orchidaceae). Both deceptive and dual mechanisms, with the latter combining floral fragrance rewards and food deception, have been described for the allogamous Neotropical *Vanilla* species. The aim of this study was to better understand the pollination mechanism of the Neotropical species *Vanilla hartii*, which we expect to be nectar-rewarding. Four *V. hartii* populations were selected within our study area in southwest Costa Rica. We tested for nectar presence, analysed nectar composition, and examined floral fragrances. Furthermore, we observed floral visitors and documented their behaviour, quantified fruit set for two years, and compared morphological traits of both *V. hartii* flowers and their visitors. All sampled flowers contained nectar that averages 34.79 % sugar, dominated by sucrose. We observed *Euglossa* spp. entering the labellum to search for nectar, indicated by their extended proboscis and prolonged visits, some of which exited the labellar tube with pollen attached to their scutellum. Combining our behavioural and phytochemical data, we demonstrate the presence of a nectar-based pollination mechanism in the genus *Vanilla*, that shows a higher natural fruit set compared to deceptive, allogamous *Vanilla* species. We provide an overview of the pollination mechanisms known so far to give initial insights into the potential evolution of reproductive strategies within this commercially important orchid genus. It may function as a tool for the development of integral conservation policies, and to further identify current knowledge gaps.

4.2 INTRODUCTION

Pollination services provide a wide range of benefits in terms of global food security, biodiversity conservation, and ecosystem stability (Porto et al., 2020; Potts et al., 2016; van der Sluijs, 2020). Approximately 85-90 % of all flowering plant species, including about 60 % of food crops, require animal vectors for pollination (Klein et al., 2007; Ollerton, 2021; Ollerton et al., 2011). Plants have developed various pollinator attraction strategies, involving a range of sensory signals (e.g., flower shape, colour, fragrances), the enticement of floral rewards (e.g., nectar, pollen, oils, resins) or deception (e.g., based on sex, food, brood-site) (van der Pijl 1966; Dafni 1984; Ackerman 1986; Jersáková et al. 2006; Wright & Schiestl 2009; Schiestl 2011). Food deception is the most common form of deceit in the orchid family (Orchidaceae), with about one third of the orchid species displaying mimicry strategies to deceive their pollinators (van der Pijl 1966; Shrestha et al. 2020).

Despite being a highly valued spice used in several industries, scant documentation exists on the natural pollination and phenology of species belonging to the orchid genus *Vanilla* Plum. ex Mill. (1754). In commercial plantations, flowers of the predominantly cultivated species *Vanilla planifolia* Jacks. ex Andrews are hand-pollinated, a technique that was established due to the lack of natural pollinators in cultivation areas outside Mesoamerica, the native region of *V. planifolia* and other species producing fragrant fruits (*Vanilla* sect. *Xanata*) (Bory et al. 2008a; Karremans et al. 2020). Manual pollination, however, reduces gene flow, as pollen is placed on the stigma of the same flower. Furthermore, given that vanilla is clonally propagated, this crop species has passed through a genetic bottleneck since its human dispersion as a cash crop (Bory et al. 2008b; Lubinsky et al. 2008). The resultant narrow genetic base has been negatively affecting crop vitality and survival for years. In their natural habitat, *Vanilla* species are pollinated by means of autogamy or animal vectors (Bateman et al., 2004; Gigant et al., 2016; Lubinsky et al., 2006; Pansarin, 2016; Pansarin and Pansarin, 2014; Rodolphe et al., 2012; van Dam et al., 2010). Outcrossing via animal vectors leads to greater gene flow within and among populations (Gamba and Muchhala, 2020; Ollerton, 2021). Hence, wild vanilla populations, which have passed through a long evolutionary history across diverse environments (Andriamihaja et al., 2020; Flanagan and Mosquera-Espinosa, 2016; Serna-Sánchez et al., 2021), contain a diverse gene pool holding interesting traits for crop improvement (Chambers et al., 2021). Their protection is of high-priority to avoid genetic depletion, which may be exacerbated by current threats related to habitat alteration, illegal extraction from the wild, and climate change (Goettsch et al. 2021), with the latter potentially provoking plant-pollinator mismatches (Dalsgaard, 2020; Forrest, 2015). Pollination

studies are essential to gain insights into plant-pollinator interactions of *Vanilla* species, thereby providing key information to develop integral conservation policies including this precious commodity.

Orchid bees (Apidae: Euglossini) have been suggested as effective pollinators of allogamous Neotropical *Vanilla* species (e.g. Ackerman 1983a; Lubinsky et al. 2006; Householder et al. 2010; Soto Arenas & Dressler 2010; Pansarin & Pansarin 2014; Anjos et al. 2016; Watteyn et al. 2021), attracted by means of deceptive or dual mechanisms. For example, the hypothesized dual attraction of *Eulaema cingulata* to *V. pompona* flowers involves bees that are both attracted to the flowers to collect floral fragrances, and duped into entering the nectarless flowers to seek food. The latter behaviour results in successful pollen removal (Watteyn et al. 2021). Through a combination of visual flower inspection, nectar extraction and analysis, and behavioural observations of floral visitors, the authors showed that *V. pompona* does not offer nectar rewards. In contrast to prior studies, Pansarin (2021) stated that *V. pompona*, as well as other Neotropical *Vanilla* species, offer nectar rewards. The study was based on visual inspection of sugar traces rather than measurements of nectar volume and composition, and no floral visitors or pollination events were observed. These data are, however, essential to correctly asses any pollination mechanism. Available nectar volume, concentration and composition are expected to reflect the energy requirements and nectar consumption mode of the plant's effective pollinators (Heinrich and Raven, 1972; Kevan and Baker, 1983; Shrestha et al., 2020). The suggested pollinators of the Neotropical *Vanilla* species, mainly euglossine bees, have high energy requirements due to their long-distance foraging behaviour (Kato et al. 1992; Suarez et al. 2005; Pokorny et al. 2014) and large body size (Nicolson and Thornburg, 2007; Rodríguez-Riaño et al., 2014). Euglossini are known to forage nectar resources that are easily measurable (3.7-51.1 µl) and have average sugar concentrations of 25.5-38 % (Roubik et al. 1995; Hentrich 2008). As sufficient nectar resources generate consistent visits and effective pollinator services (Ackerman et al. 1982; Hentrich 2008), we expect that true nectar-rewarding *Vanilla* species offer quantifiable nectar to its pollinators and are more frequently visited compared to their congeneric nectarless species.

This study aims to reveal the pollination mechanism of the Neotropical species *Vanilla hartii* Rolfe, suggested to be a nectar-rewarding species (Soto Arenas & Dressler 2010; Pansarin 2021). Behavioural observations are combined with phytochemical data to link the potential presence of floral rewards with pollinator attraction and pollination events. Our study sites are on the Osa Peninsula in southwest Costa Rica, a biodiversity hotspot (Kohlmann et al., 2010) where several *V. hartii* populations can be found. We hypothesize that (i) the pollination mechanism is based on

floral nectar rewards, (ii) the fruit set is higher compared to *Vanilla* species lacking food rewards, and (iii) natural reproduction is achieved by animal vectors belonging to the Euglossini tribe. A comparative overview of putative and confirmed pollination strategies among the *Vanilla* species is presented and discussed to assess potential evolutionary trajectories within this genus.

4.3 MATERIALS AND METHODS

4.3.1. Study area and species

The Osa Peninsula includes protected areas (national parks, forest reserves, wildlife refuges), that are surrounded by cattle ranches, agriculture and human settlements. The average annual precipitation ranges from 2,500 to 6,000 mm, and the average annual temperature is 25 °C (Holdridge, 1967; Kappelle et al., 2015). The sites with *V. hartii* populations are Bambu (8.5267 N, 83.3254 W; 80 m a.s.l.), Bangeas (8.6767 N, 83.5195 W; 119 m a.s.l.), Los Mogos (9.7331 N, 83.4119 W; 117 m a.s.l.), Piro (8.4102 N, 83.3307 W; 166 m a.s.l.) (Fig. 4.1). All four study sites are in old-growth forest, where *V. hartii* grows on understory trees, in deep shade.

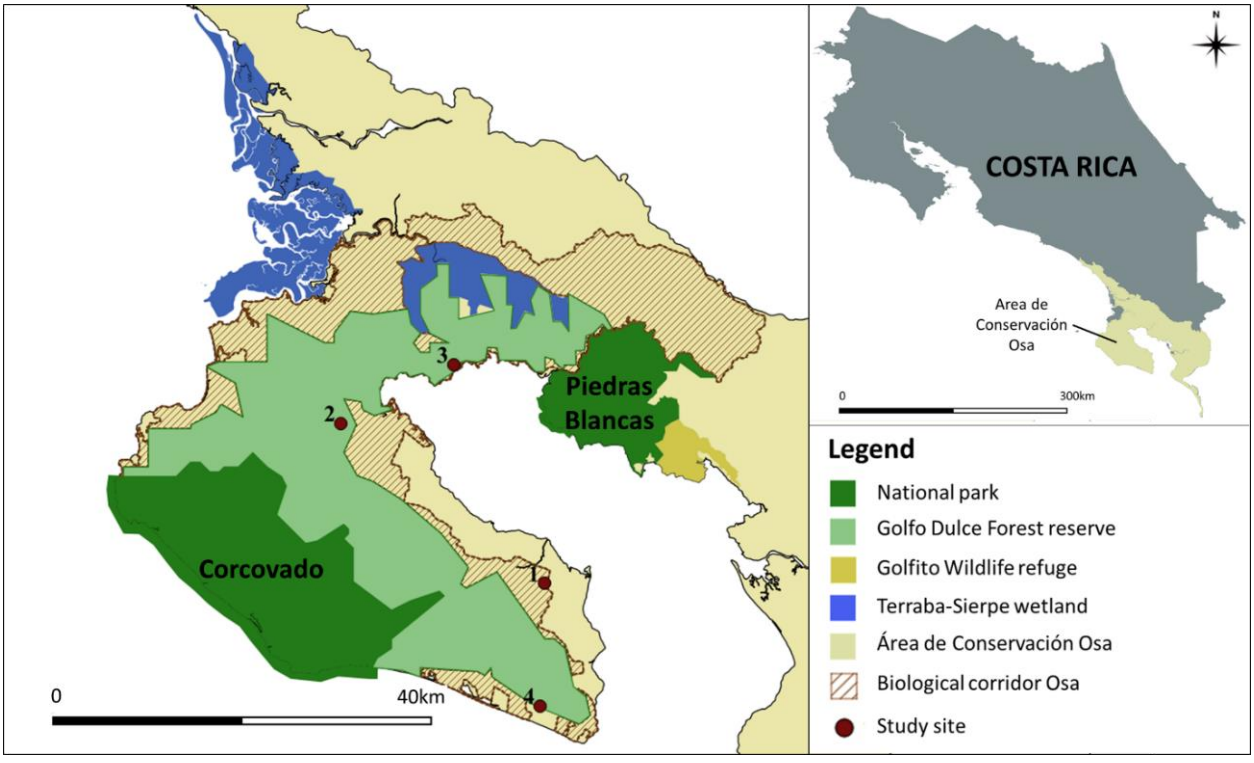

Fig. 4.1. *The Osa Peninsula, our study area in southwest Costa Rica, with indications of the four study sites with a Vanilla hartii population: (1) Bambu, (2) Banegas, (3) Los Mogos, (4) Piro.*

Vanilla hartii, featured on the IUCN list as an endangered species (Wegier et al. 2020), has a distribution from Mexico to Brazil (Soto Arenas & Dressler 2010; Ferreira et al. 2020; Karremans et al. 2020; Watteyn et al. 2020). The inflorescence is a short raceme with 5-8 flowers, of which 1-3 flowers open simultaneously and last for one day. Flowers consist of three greenish sepals, two lateral white petals, a white lip with greyish to brown lines, and a white, penicillate callus at the lip apex (Fig. 4.2-A). The lip or labellum is partially fused to the column, which bears a single ventral stigma, separated by the rostellum from the anther holding two pollinia-like pollen masses (henceforward pollen) that are deposited as smears. The margin is crenulated at the apex. The fruits have a characteristic vanilla aroma when ripe (Soto Arenas & Dressler 2010).

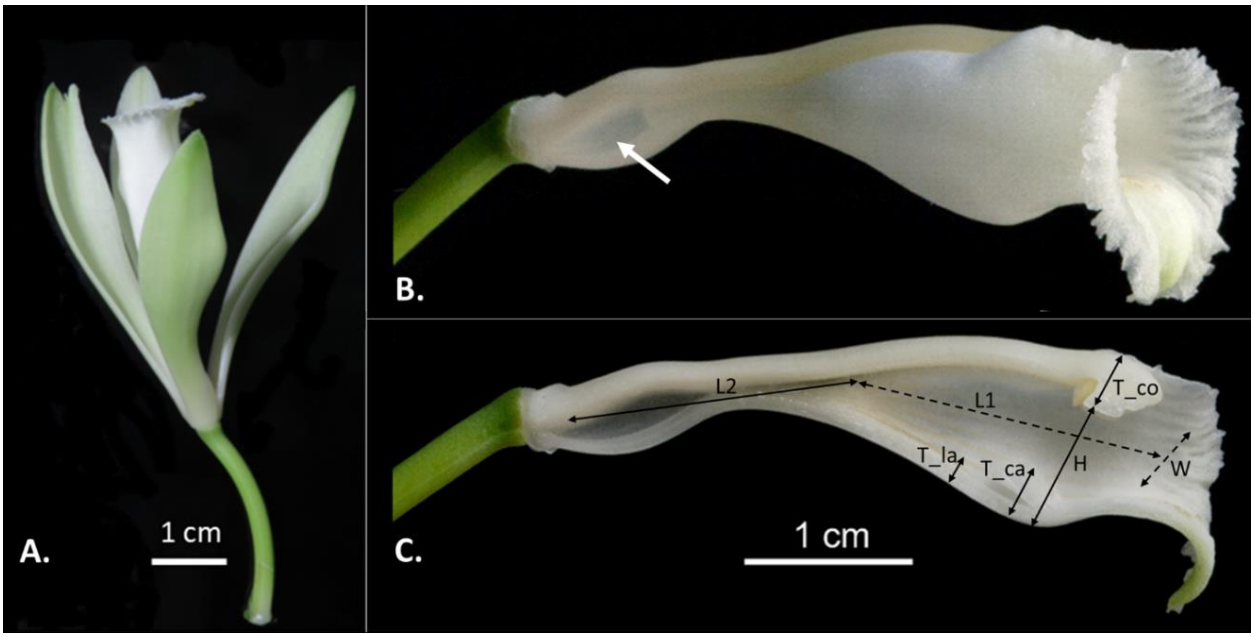

Fig. 4.2. *Vanilla hartii flower (A), and V. hartii flower with removed sepals and petals to indicate the nectary (white arrow) (B) and the measured floral traits: L1 = length of labellar tube, L2 = length of pedicel, T_co = thickness of column at anther height, T_la = height of labellar keels, T_ca = height of labellar callus, H = height of labellar tube below anther, W = width of labellar tube (C). (vouchers at JBL)*

4.3.2 Floral rewards

Nectar

During the flowering period of December 2020 - February 2021, we bagged a total of 5 flowers per population a day before anthesis to avoid nectar removal by floral visitors, and inspected the presence of nectaries (Fig. 4.2-B). Next, we collected 35 nectar samples from additionally bagged flowers (Bambu: 10, Bangeas: 8, Los Mogos: 9, Piro: 8) using 5 µL microcapillary tubes (Drummond Scientific Company, Broomal, PA, USA), a non-destructive sampling method whereby nectar is drawn into the tubes by capillary action (Morrant et al., 2009). Nectar sampling

was carried out at 0600 h, immediately after anthesis. We quantified nectar volume and analysed nectar sugar content by high-performance liquid chromatography (HPLC) in an Agilent 1260 Infinity system (Santa Clara, USA) with refractive index detector (G1362A). Sample preparation for injection consisted of diluting an aliquot of nectar sample from individual flowers in ultrapure water, type 1 (Milli-Q®, Millipore Corporation, Burlington, USA), using dilution ratios ranging from 5- to 58-fold as needed. Sugar separation was achieved by injecting 9 to 15 µL of sample solution into a Hi-Plex Ca2+ column (Agilent, Santa Clara, USA) (300 × 7.7 mm, 8 µm) at 45 °C, in isocratic mode with a flow of 0.5 mL/min. Adequate separation occurred in 25 min chromatographic runs.

The Kruskal-Wallis test and Dunn's post-hoc tests with multiple comparison correction using the Benjamini-Hochberg adjustment to control the False Discovery Rate was used to compare nectar volumes among the populations and to compare the proportions of each of the identified nectar sugars to the total sugar composition. Nectar sugar composition was compared among the populations and flower individuals using non-metric multidimensional scaling (NMDS), with Bray-Curtis dissimilarity calculation and two dimensions (Oksanen et al., 2019).

Floral volatile compounds

Floral fragrances of *V. hartii* flowers were collected using static headspace sampling (Tholl et al., 2006). Sampling took place at three populations (Bambu, Banegas, Piro), during the flowering period of December 2019 - February 2020. We sampled from 0700 h to 1100 h, which conforms to the activity peak of potential pollinators (Ackerman 1983b; Montalvo & Ackerman 1986; Armbruster & McCormick 1990). The single-use scent trap consisted of a glass tube (2.4 mm ID, 3.5 cm length), filled with 20 mg of carbon and 20 mg of Tenax GC, and of which both ends are plugged with glass wool. We bagged an inflorescence with open flower(s) using a plastic, scentless oven bag (Reynolds Kitchens) that was closed at the top with a plastic strip. The scent traps, conditioned with 5 mL of hexane, were connected with the tenax-side to the bag and the carbon-side to the electrical vacuum pump via Tygon tubing (ID 3.3 mm). For three hours, air was extracted through the scent trap at 2.5 L min^{-1}. We subsequently eluted the scent traps with 2 mL of hexane into sealed, 2 mL auto-sampler vials (Agilent Technologies). Ambient air was collected simultaneously as control samples to identify background contamination.

Samples were analysed via gas chromatography-mass spectrometry (GC-MS) in an Agilent 7890B GC fitted with a 30 m x 0.25 mm x 0.25 µm HP-5 Ultra Inert column coupled to an Agilent 5977A mass spectrometer (Agilent Technologies). We used the same methodology for data analysis and processing, and compound identification and attractiveness as described in Hetherington-Rauth & Ramírez (2016).

4.3.3 Floral visitors and their behaviours: identifying potential pollinators

We observed *V. hartii* flowers and their potential visitors using CANON SX60 HS cameras, located at a minimum distance of three meters from the flowers to avoid potential disturbance. Observations took place during two flowering periods (December 2018 - January 2019 and December 2019 - February 2020), from 0600 h (anthesis) to 1300 h (when they start to wither). Each of the four populations flowered for about two to three weeks, but with varying starting dates. This results in a complete flowering period of two to three months during the dry season, which conforms with previous observations by Soto Arenas & Dressler (2010). In Costa Rica, *V. hartii* also has a short flowering period from August to September, but no camera observations were carried out during this period due to logistical constraints provoked by the heavy rains. Cameras were activated every time a potential visitor approached the flowers. We subsequently analysed the video footage to distinguish floral visitors from potential pollinators based on their behaviours: (i) only approaching, (ii) landing on tepals or labellum to probe, (iii) landing on labellum to search for nectar, (iv) entering labellum to search for nectar, thereby potentially or effectively removing the pollen. The floral visitors that were observed removing the pollen of *V. hartii* flowers were identified and determined by sex (Roubik and Hanson, 2004). Moreover, to test for autogamy, twenty inflorescences with newly-opened flowers, of which no pollen was deposited on the stigma, were covered with an insect proof nylon bag until floral senescence (Scaccabarozzi, et al. 2020). This experiment took place in two populations (Bambu and Banegas) at the beginning of the second flowering period.

To determine potential pollinator species based on a morphological fit between bee body and flower traits, chemical baits (eucalyptol, eugenol, methyl salicylate) shown to be effective Euglossini attractants (Ackerman, 1989; Dressler, 1982; Roubik and Hanson, 2004; Williams and Dodson, 1972) were located haphazardly during one morning (0800 h - 1100 h) within each *V. hartii* population (during the flowering period of December 2020 - February 2021). Attracted individuals were identified and the following bee body traits were measured and compared with *V. hartii* floral traits to assess a potential morphological-fit-to-flower structure: total body length, mesosoma height and width, and proboscis length. Previous research has shown that the “An-Cn” gap of *V. pompona* flowers, i.e. the gap between the labellar tube entrance at the apex of the anther (An) and the labellar tube measured at the height of the callus below the rostellar flap (Cn), plays an important role in predicting the goodness-of-fit with the mesosoma height of its pollinator *Eulaema cingulata* (Watteyn et al. 2021). Since *V. hartii* has a similar floral structure as *V. pompona*, we used the same prediction to select potential *V. hartii* pollinators. The following traits

were measured on 40 *V. hartii* flowers (10 per population): width (W) and length (L1) of the labellar tube, height of the labellar tube below the anther (H), thickness of the column at anther height (T_Co), height of the labellar callus (T_Ca), height of the labellar keels (T_La), and length of the pedicel (L2) (Fig. 4.2-C). The derived variables An and Cn were estimated using abovementioned traits (An = H - T_La; Cn = H - T_Ca). Moreover, captured *Euglossa* individuals were placed together with *V. hartii* flowers in a flying cage (Fig. A3.1) to observe their behaviour during one hour. Individuals that entered the labellum and exited with pollen attached to their scutellum were identified at species level.

4.3.4 Pollination success

We counted the number of inflorescences per population, the number of flowers per inflorescence, and at the end of each flowering period we recorded the number of developed fruits per inflorescence. Fruit set was then calculated at the inflorescence level and compared among (a) the four populations using the non-parametric Kruskal-Wallis test and Dunn's post-hoc tests with Benjamini-Hochberg adjustment to control the False Discovery Rate, and (b) the two flowering periods using the non-parametric Mann-Whitney U test.

4.4 RESULTS

4.4.1 Floral rewards

The average nectar volume (± SD) per flower was 1.85 ± 1.07 µL, ranging from 0.26 to 4.46 µL. No significant differences in nectar volumes were found among the four populations (H = 3.1, P = 0.38) (Fig. 4.3-A). Sugar concentration (± SD) averaged 34.79 ± 6.01 %, and we identified four nectar sugars (Fig. A3.2), that significantly differed among each other in their contribution to the nectar sugar composition (H = 102.0, P < 0.01). The disaccharide sucrose (83.64 ± 23.01 %) represented the highest proportion, followed by the two hexoses, glucose (8.92 ± 14.20 %) and fructose (5.91 ± 7.20 %), and the trisaccharide raffinose (1.52 ± 2.43 %) (Fig. 4.3-B). No significant differences in nectar sugar composition were found among the four populations (ANOSIM R = 0.06, P = 0.07) but substantial variability exists among the flowers within a certain population (Fig. 4.3-C). Based on the results of the GC-MS analysis, we identified two floral volatile compounds, 1,8-cineole and methyl salicylate. These two compounds were found in trace quantities (< 2 % of the total peak area) in respectively three and four of the ten samples (Fig. A3.3). The other observed peaks were identified as contamination as they were present in both sample and control.

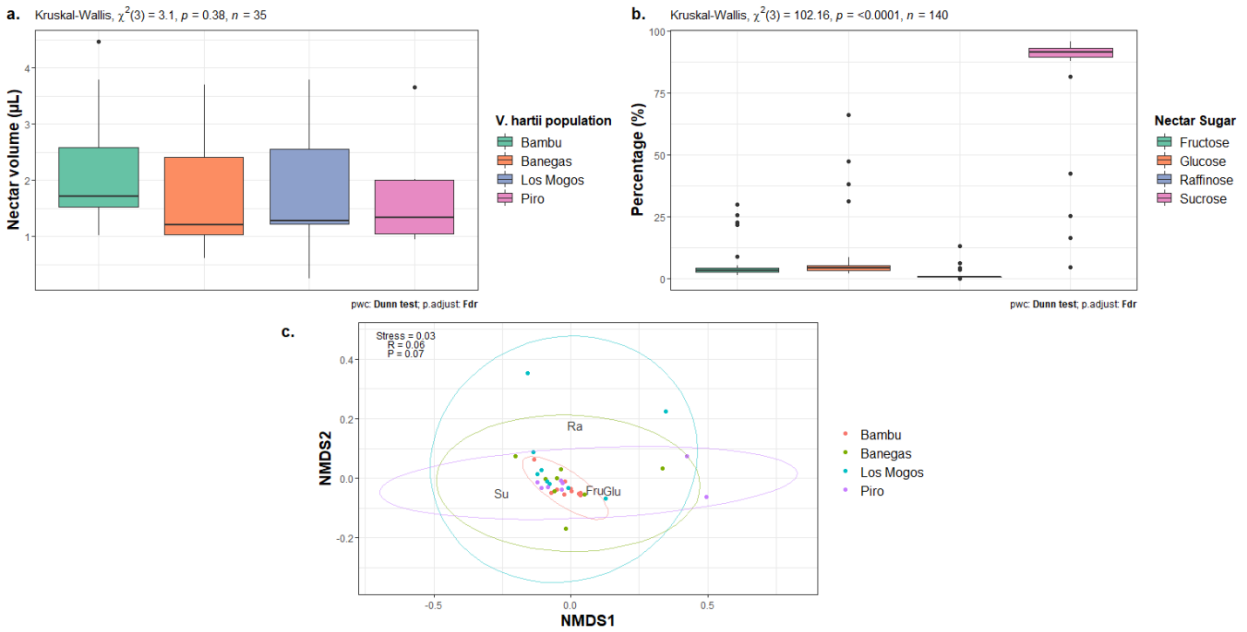

Fig. 4.3. *Comparison of (A) the nectar volumes between the four V. hartii populations, (B) the contribution of each of the identified nectar sugars present in V. hartii nectar and (C) the V. hartii nectar sugar composition among the four populations using non-metric multidimensional scaling, with Fru = fructose, Glu = glucose, Ra = raffinose, Su = sucrose.*

4.4.2 Floral visitors and their behaviour: identifying potential pollinators

All but two of 58 individuals observed approaching *V. hartii* flowers were bees (Apidae). More specifically, 27.6 % were Euglossini belonging to the genera *Euglossa* (24.2 %) and *Eulaema* (3.4 %), and 69 % were *Trigona* (Meliponini). The two other approaches were by hummingbirds (*Phaethornis*). The approaching *Eulaema* and about 78.6% of the approaching *Euglossa* subsequently landed on the flowers. They all landed on the labellum and displayed a nectar search behavior indicated by their extended proboscis when exiting the flower (Video 4.1). Only certain *Euglossa* individuals (n = 9) fully entered the labellum to search for nectar, of which four were observed with pollen attached to their scutellum when exiting the labellar tube (Video 4.2). About 80 % of the Meliponini (n = 40) landed on the flower, i.e. the sepals, petals, or labellum edge. They all showed the same probing behaviour of touching the flower surface with their proboscis or antennae. Both hummingbirds inserted their beaks into the labellum, and pitched the pedicel from beneath, indicative of nectar search (Video 4.1). No pollen removal by any *Eulaema*, Meliponini or hummingbird species was observed.

The autogamy test resulted in zero fruit development, confirming vector-mediated pollination. Only *Euglossa*, both male (n = 7) and female (n = 2), fully entered the labellar tube, of which three male and one female successfully removed the pollen. The average time (± SD) that individuals

spent inside the labellar tube was 11.8 ± 2.5 s. As they exited, an extended proboscis was observed, indicative of nectar search. A list of potential *V. hartii* pollinator species was obtained by performing a bee-to-flower trait assessment with euglossines caught at baits from the four populations (Fig. 4.4). All except *Euglossa imperialis*, *Euglossa cognata* and *Eulaema* have a mesosoma width (± SD) smaller than the width of the floral tube (6.00 ± 0.51 mm), but only the following *Euglossa* species have a mesosoma height (± SD) that fits in the "An-Cn" gap: *E. allosticta*, *E. championi*, *E. cybelia*, *E. flammea*, *E. mixta*, *E. tridentata*, *E. villosiventris*. All except *Euglossa allosticta* and *E. flammea* have a proboscis length that equals or is smaller than the nectar tube length (± SD) of *V. hartii* flowers (15.42 ± 0.51 mm). These species would need to enter the labellum to reach the nectar reward. On the contrary, *Euglossa allosticta* and *E. flammea* have a longer proboscis (23.4 ± 3.1 mm and 35.1 ± 1.9 mm respectively), and may not necessarily need to enter the labellar tube to reach the nectar. Moreover, *Euglossa cybelia* and *E. tridentata* were observed entering the labellar tube and effectively removing *V. hartii* pollen when placed in the flying cage (Fig. A3.4).

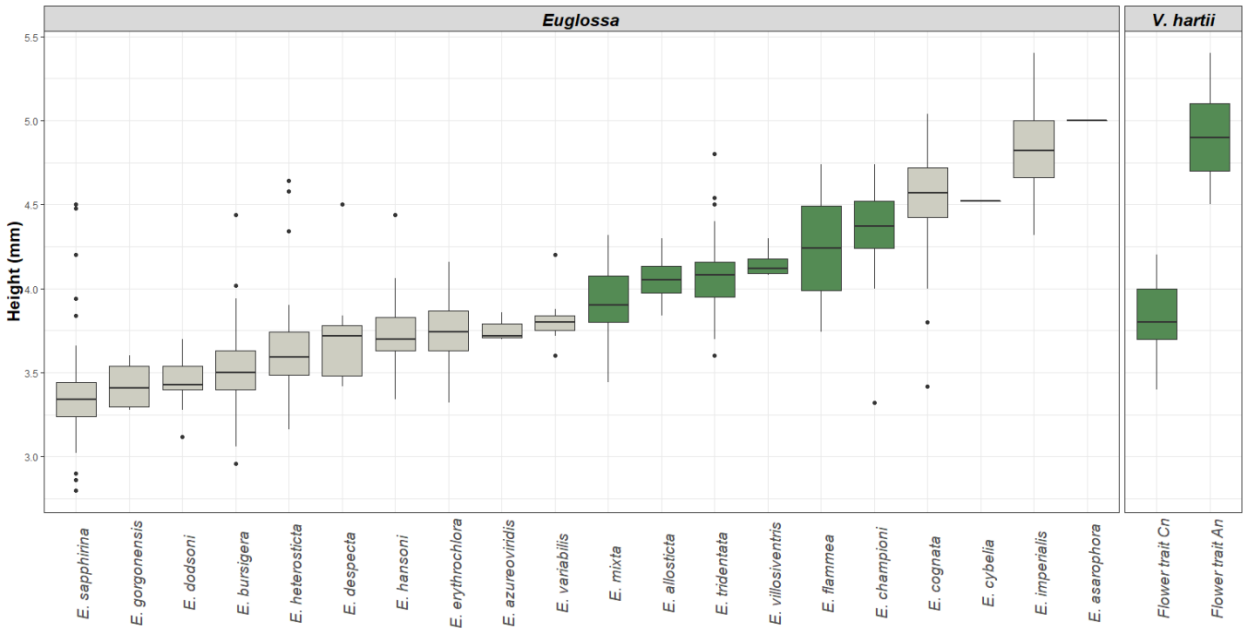

Fig. 4.4. *Comparison of the mesosoma heights (mm) of the caught Euglossa spp. with the flower morphological traits "An" (mm) and "Cn" (mm) of Vanilla hartii. The potential pollinator species, i.e. the Euglossa species with a mesosoma width smaller than the diameter of the labellar tube and a height that fits into the "An-Cn" gap, are shown in green.*

4.4.3 Pollination success

No significant differences in fruit set (H = 4.62, P = 0.20) were found among the populations Piro (2019: 12.9 ± 15.2 %; 2020: 8.5 ± 13.7 %), Banegas (2019: 11.2 ± 16.0 %; 2020: 10.8 ± 15.2 %), Bambu (2019: 6.6 ± 10.9 %; 2020: 7.2 ± 11.4 %), and Los Mogos (2019: 3.6 ± 7.0 %; 2020: 6.2 ± 15.1 %), and neither between the observed flowering periods (W = 3660, P = 0.49) (respectively 9.4 ± 13.9 % in 2019 and 8.3 ± 13.8 % in 2020).

4.5 DISCUSSION

Vanilla hartii rewards its floral visitors by offering extractable amounts of sugar-rich nectar. Previous *Vanilla* pollination studies have described pollinator attraction via floral fragrances (Soto Arenas & Dressler 2010), food deception (Nielssen & Ackerman unpubl.; Pansarin & Pansarin 2014; Pansarin et al. 2014; Anjos et al. 2016), or a combination of both (Watteyn et al. 2021). We present novel insights into the adaptation of *Vanilla* species to food-seeking pollinators, whereby the nectar-rewarding strategy tends to be more efficient and to result in higher fruit set – as observed in other plant species by Scopece et al. (2010) and Tremblay et al. (2005).

4.5.1 Nectar-rewarding pollination mechanism

The GC-MS identified two floral volatile compounds, 1,8-cineole and methyl salicylate, known as general Euglossini-attractants and common odour constituents of several Euglossini-pollinated orchid species (e.g. Dodson et al. 1969; Williams & Whitten 1983; Roubik & Knudsen 2017). These volatile compounds, however, were only present in a few samples, and in trace quantities. Conversely, nectar was present in all sampled flowers. Nectar volume, sugar concentration and composition did not vary among the four populations, but did vary among individual flowers. This inter-individual variability probably stimulates bees to forage for multiple flowers in order to fulfil their energy demand (Borrell, 2007), thereby promoting outcrossing necessary for genetic variation, which in turn favours plant fitness and survival (Pyke, 2016; Pyke et al., 2020). An overall dominance of the disaccharide sucrose was observed, which is typical for bee-pollinated flowers (Nicolson 2007; Patiny 2011; Parachnowitsch et al. 2019; Scaccabarozzi et al. 2020). Furthermore, nectar sugar concentrations in *V. hartii* conform with previous studies (Ackerman et al. 1982; Roubik et al. 1995; Hentrich 2008) demonstrating that Euglossini exploit flowers with nectar containing sugar concentrations between 25 and 40%, as this viscosity provides an optimal energy intake rate by means of their typical suction feeding style (Pokorny et al., 2014).

Male Euglossini are known for their fragrance collection behaviour, displayed on the flowers they pollinate. More precisely, specific mixtures of floral volatile compounds selectively attract the plant's pollinators (Dodson et al., 1969; Dressler, 1982; Eltz and Lunau, 2005; Hetherington-Rauth and Ramírez, 2016; Ramírez et al., 2011; Williams and Dodson, 1972). Even though we identified two *Euglossa*-attractive compounds, no fragrance collection behaviour on the sepals, petals or labellum of *V. hartii* was observed - a sharp contrast with the observation of continuous fragrance collection on the flowers of the sympatric *V. pompona* (Watteyn et al. 2021). Instead, all the *Euglossa* individuals visiting *V. hartii* flowers were observed searching for nectar in the labellum. Certain individuals were able to fully enter the tube formed by the labellum; four of which were observed with pollen attached to their scutellum when exiting the labellar tube. This nectar feeding behaviour is consistent with the observed prolonged visits and an extended proboscis. The specific role of the floral fragrances could be examined through flower manipulation experiments (e.g. removing floral scents or by obscuring visual cues). Nectar is rarely detectable at long distances and sugar concentration or composition cannot be perceived from a distance (Pyke et al., 2020). Therefore, the volatile compounds may function as long-distance attractants, coupled with the white labellum which stands out in the dark understory of old growth forests.

4.5.2 Floral visitors and potential pollinators

The most common floral visitors were stingless bees (*Trigona*), followed by orchid bees (*Eulaema*, *Euglossa*) and occasionally hummingbirds. The orchid bees and hummingbirds inspected the floral tube in search of nectar. Only median- or small-sized *Euglossa* species were able to fully enter the tube formed by the labellum. A subset of these bees were observed with pollen attached to their scutellum when exiting the labellar tube, acting as effective *V. hartii* pollinators. The others foraged nectar, but were not able to make contact with the reproductive floral structures, and may be regarded as nectar thieves (Bronstein et al., 2017; Irwin et al., 2010). Both male and female *Euglossa* were observed visiting and entering the labellum in search of nectar, which is consistent with previous studies demonstrating the attraction of both sexes towards nectar-rewarding plant species (e.g., López-Uribe et al. 2008; Rocha-Filho et al. 2012). Due to their swiftness when exiting the flowers and the height at which the flowers were located (> 3 m), we were not able to physically capture these individuals. Nevertheless, the digitally captured pollinators were assessed morphologically, identified using Roubik & Hanson (2004), and compared to Euglossini captured at baits.

Based on the species that we identified as potential pollinators, we conclude that *V. hartii* is possibly pollinated by a range of functionally similar *Euglossa* species. Five of the species caught (*Euglossa championi*, *E. cybelia*, *E. mixta*, *E. tridentata*, *E. villosiventris*) had a mesosoma height that allows them to fit into the labellar tube ("An-Cn" gap) and a proboscis length that is similar to the nectar tube length of *V. hartii* flowers. Both morph traits are required for pollen removal and deposition to occur in this orchid species. Such an interpretation conforms with previous studies demonstrating the link between pollinator proboscis length and floral nectar tube length (e.g. Inouye 1980; Klumpers et al. 2019; Naghiloo et al. 2021). When placed together in an enclosed space, males of *E. cybelia* and *E. tridentata* were observed successfully entering the labellar tube and effectively removing pollen of *V. hartii*. As such, we identified the same potential pollinator species from both the morphological assessment as well as experimental allocation of *Euglossa* bees inside a flying cage with *V. hartii* flowers.

4.5.3 Mapping vanilla pollination strategies

Within each subgenus and section of *Vanilla*, we grouped species according to their pollination strategy and added their reproductive success (Table 4.1). So far, the pollination biology of 16 species is fairly well understood (exclusively including studies with concise data on pollination events, fruit set and identification of potential pollinators), which corresponds to only ± 13% of the known *Vanilla* species (Karremans et al. 2020). Yet, this overview allows us to gain some first insights into potential evolutionary trends of *Vanilla* pollination strategies and pollinator species. As documented in other orchid genera (Tremblay et al., 2005), autogamous *Vanilla* species tend to have a higher reproductive success compared to allogamous species. Ortiz-Barney & Ackerman (1999) discussed various drivers of autogamy, of which reproductive assurance was the most common one. As observed by Gigant et al. (2016), however, autogamy seems to be more of an ancestral feature within the *Vanilla* genus (*V. mexicana* and *V. palmarum* group), raising questions about evolutionary directions between allogamous and autogamous mating systems.

Species pollinated by animal vectors seem to display different strategies, such as rewarding, deceptive or dual attraction (floral fragrance and food lure) mechanisms. As far as we know, all but one allogamous *Vanilla* species are pollinated by Apidae (Hymenoptera), and there seems to be some phylogenetic specificity. To date, only genera of euglossine bees (*Euglossa*, *Eulaema* and *Eufriesia*) have been shown to successfully pollinate members of *Vanilla* subg. *Xanata* sect. *Xanata* - which includes *V. planifolia* and all other fragrant *Vanilla* species. Whereas the genus

Allodape (Apidae: Allodapini) has been reported as pollinator of *Vanilla* subgen. *Xanata* sect. *Tethya*, and the genus *Epicharis* (Apidae: Centridini) has been shown to pollinate a member of *Vanilla* subg. *Vanilla*. Only *V. siamensis* of subg. *Vanilla* sect. *Tethya* is pollinated by bees other than Apidae: *Thrinchostoma* spp. (Halictidae: Halictini).

Even though there are large information gaps regarding the pollinators of many *Vanilla* species, the available studies suggest the presence of multiple pollinator groups and pollination mechanisms in the genus, potentially with phylogenetic as well as biogeographic specialization. Members of *Vanilla* sect. *Xanata* are endemic to the Neotropics, and so are the Euglossini that have been shown to pollinate some of them. Species of *Vanilla* sect. *Xanata* not only produce fragrant fruits, many are also known for their fragrant flowers (Soto Arenas & Dressler 2010; Karremans et al. 2020) that possibly exploit the fragrance-seeking behaviour of male euglossine bees. Hence, nectarless *Vanilla* species with fragrant flowers may actually display a dual mechanism, as has been hypothesized for *V. pompona* (Watteyn et al. 2021). The present study confirms the presence of a nectar-rewarding species within this group, as suggested by Soto Arenas & Dressler (2010) and Pansarin (2022). Phylogenetic reconstructions (Bouetard et al. 2010; Soto Arenas & Dressler 2010; Azofeifa-Bolaños et al. 2017) show that *V. hartii* has an isolated position as sister to most of the other, seemingly deceptive, members of *Vanilla* section *Xanata*. Various orchid pollination studies (e.g. Cozzolino et al., 2005; Jersáková et al., 2006; Stípková et al., 2020) suggest that deceptive mechanisms evolved to avoid inbreeding depression, as it reduces the number of pollinator visits per inflorescence due to the lack of a reward. This may may explain the phylogenetic distinct position of the nectar-rewarding *V. hartii*. We highly recommend further research to link the evolutionary divergence within this section with observed differences in pollinator mechanisms.

Based on the current limited information, we see great value in future studies that (a) combine observational data (pollinator species and their behaviours, pollination success, flower shape) and phytochemical flower analysis (presence of nectar and floral volatile compounds) to further understand possible evolutionary trends among the different *Vanilla* species, (b) examine the potential associations between vanilla bean size, flower to bean ratio (fruit set) and pollination strategy, and (c) compare the habitat requirements, phenology and pollinator species in order to reveal further ecological adaptations that may have driven the evolution of different pollination strategies within the genus. As such, we will be able to better frame the complex *Vanilla*-pollinator interactions within a wider evolutionary context.

4.6 CONCLUDING REMARKS ON THE POLLINTION OF FRAGRANT *VANILLA* SPECIES

The majority of the fragrant *Vanilla* species (*Vanilla* sect. *Xanata*), comprising species with potential for cultivation, seem to depend on animal vectors for pollination. Insect-mediated pollination benefits outcrossing, promoting the long-term viability of wild populations and the development of interesting agronomic traits to be used for crop improvement, such as aromatic profile diversification, pest resistance or climate change adaptation (Havkin-Frenkel & Belanger 2010, 2018; Ranadive 2010; Maruenda et al. 2013; Azofeifa-Bolaños et al. 2014; Flanagan & Mosquera-Espinosa 2016). The conservation of wild vanilla populations and their natural interactions is therefore crucial to safeguard the future of this high-value crop. Pollination studies provide important insights to define proper *Vanilla* conservation strategies, and to inform about possible agro-ecological practices involving natural pollination. Our comparative overview, however, indicates a large knowledge gap in the reproductive biology of this genus. We identify three necessary steps for developing effective conservation policies with integration of plant-pollinator interactions: (a) conducting and implementing collaborative and integral *Vanilla* pollination studies, comparing pollinators and pollination strategies among different growing areas, (b) characterizing *Vanilla* and pollinator species habitat, through the combination of *in situ* observations and modelling techniques that define current and potential distributions (i.e. subjected to climate change, habitat destruction or other potential threats), and (c) identifying high-importance conservation areas for both *Vanilla* and pollinator species.

Table 4.1. *Overview of the proposed pollination strategies, pollinators, and reproductive success (fruit set (%)) of Vanilla species studied so far, grouped by subgenus and section according Soto Arenas & Cribb (2010) and Karremans et al. (2020). All pollinator species are bees belonging to the Apidae family, except for V. saimensis, which is pollinated by Halictidae.*

Species	Pollination strategy	Pollinator	Fruit set	References
Vanilla* subg. *Xanata* sect. *Xanata				
Vanilla hartii Rolfe	Allogamous - Nectar-rewarding	Euglossini: *Euglossa* spp.	3.6-12.9	Present study
Vanilla pompona Schiede[1]	Allogamous - Dual	Euglossini: *Eulaema cingulata*	0.9-2.4	Watteyn et al. 2021
		Euglossini: *Eulaema meriana*		Lubinsky et al. 2006
				Householder et al. 2010
		Euglossini: *Eulaema nigrita*		Ackerman 1983a
Vanilla bahiana Hoehne[2]	Allogamous - Food-deceptive	Euglossini: *Eulaema* spp.	2.4	Anjos et al. 2017
Vanilla dubia Hoehne[3]	Allogamous - Food-deceptive	Euglossini: *Eulaema nigrita*	0.4-3.2	Pansarin & Pansarin 2014
		Euglossini: *Eufriesea violacea*		
Vanilla cribbiana Soto Arenas[4]	Allogamous - Food-deceptive	Euglossini: *Eulaema* spp.	n.a.	Soto Arenas & Dressler 2010
Vanilla insignis Ames[4]	Allogamous - Food-deceptive	Euglossini: *Eulaema polychroma*	n.a.	Soto Arenas & Dressler 2010
Vanilla odorata C. Presl[4]	Allogamous - Food-deceptive	Euglossini: *Euglossa* spp.	n.a.	Karremans & Watteyn unpubl.
Vanilla planifolia Andrews[4]	Allogamous - Food-deceptive	Euglossini: *Euglossa* spp.	0.1-1.0	Soto Arenas & Dressler 2010
Vanilla trigonocarpa Hoehne[4]	Allogamous - Food-deceptive	Euglossini: *Euglossa asaraphora*	n.a.	Soto Arenas & Dressler 2010
		Euglossini: *Eulaema* spp.		Karremans & Watteyn unpubl.
Vanilla bicolor Lindl.	Autogamous		42.5-75	Householder et al. 2010
				van Dam et al. 2010
Vanilla chamissonis Klotzsch	Autogamous		15.2	Reis 2000
Vanilla palmarum Lindl[5]	Autogamous		70.3-76	Pridgeon et al. 2003
				Householder et al. 2010
Vanilla planifolia Andrews[4]	Autogamous		4.0-6.0	Hernández 1997
				Gigant et al. 2011

Vanilla* subg. *Xanata* sect. *Tethya				
Vanilla barbellata Rchb. f.[4]	Allogamous - Food-deceptive	Centridini: *Centris haemorrhoidalis*	1.0	Nielsen & Ackerman unpubl.
Vanilla bosseri	Allogamous - Food-deceptive	Allodapini: *Allodape* spp.	n.a.	Petersson, 2015
Vanilla claviculata Sw. [4]	Allogamous - Food-deceptive	Centridini: *Centris haemorrhoidalis*	5.3	Nielsen & Ackerman unpubl.
Vanilla dilloniana Correll [4]	Allogamous - Food-deceptive	Centridini: *Centris haemorrhoidalis*	0.5	Nielsen & Ackerman unpubl.
Vanilla humboltii Rchb. f.	Allogamous - Food-deceptive	Allodapini: *Allodape obscuripennis*	0.62-1.2	Gigant et al. 2016
Vanilla roscheri Rchb. f.	Allogamous - Food-deceptive	Allodapini: *Allodape variegata*	26.3	Gigant et al. 2014
		Allodapini: *Allodape rufogastra*		Gigant et al. 2014
		Anthophorini: *Anthophora*		Gigant et al. 2014
Vanilla siamensis Downie	Allogamous - Food-deceptive	Halictini: *Trinchostoma spp.*	3.6	Chaipanich et al. 2020
Vanilla griffithii Rchb. f.	Autogamous		n.a.	Cameron & Soto Arenas 2003
Vanilla* subg. *Vanilla				
Vanilla edwallii Hoehne	Allogamous - Food deceptive	Centridini: *Epicharis affinis*	n.a.	Pansarin et al. 2013
Vanilla mexicana Mill.	Autogamous		53.9	Gigant et al. 2016
Vanilla guianensis Splitg.	Autogamous		78	Soto Arenas 2003
				Householder et al. 2010
Vanilla inodora Schiede[4]	Autogamous		n.a.	Soto Arenas & Dressler 2010

[1]Dual refers to the dual pollinator attraction mechanism that combines floral fragrances and food-deception.
[2]Anjos et al. (2017) mention the observation of a visiting *Eulaema* sp., but no pollen removal or deposition was recorded.
[3]Pansarin & Pansarin (2014) provide footage of *Eulaema nigrita* collecting fragrances on the petals of *V. dubia* and *Eufriesea violacea* landing on the labellum of *V. dubia* with pollen of *Catasetum* sp., no evidence was provided of *Eulaema nigrita* or *Eufriesea violacea* acting as potential pollinators.
[4]Statements are based on unpublished data.
[5]A recent study of Pansarin (2021) reported the hummingbird *Amazilia fimbriata* as potential pollinator of *V. palmarum*. However, no pollen removal was presented, and the proposed bird-pollination would require further mechanical explanation.



5

Chapter 5

Initial insights on the cultivation potential of *Vanilla* crop wild relatives in two contrasting land use systems

Adapted from:

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Photo: The hybrid ((*Vanilla planifolia* x *Vanilla pompona*) x *Vanilla planifolia*) planted in one of the secondary forest sites as part of the field experiment, with *Cananga odorata* ("ylang ylang") as tutor tree. © Charlotte Watteyn

5.1 SUMMARY

The vanilla crop has passed through various domestication bottlenecks and associated production problems, and there is an urgent need to bring innovation within this sector. The Neotropical rainforests host, besides the crop species *Vanilla planifolia*, at least 37 fragrant *Vanilla* species, known as secondary gene pool crop wild relatives (CWR) with potentially interesting traits for crop improvement and market innovation. The aim of this study was to provide initial insights into the cultivation potential of four fragrant *Vanilla* species (*Vanilla hartii*, *V. odorata*, *V. pompona*, *V. trigonocarpa*); and this within a previously defined context of joint land sparing / sharing (SPASHA) that combines the conservation and sustainable cultivation of *Vanilla* CWRs. We established twelve experimental field sites within our study region in southwest Costa Rica, six in forested areas and six in existing agricultural areas (i.e. cacao plantations). Over a period of two years, we measured traits related to the survival, vitality, growth, and flowering of the vanilla plants. Subsequently, we developed models to predict plant performance with *Vanilla* species (CWRs, *V. planifolia*, and a disease-resistant hybrid), land use system (forest, cacao plantation), and a variety of environmental variables. Moreover, we quantified and compared the aromatic potential of mature vanilla pods among species using high-performance liquid chromatography (HPLC). The hybrid and CWRs outperformed *V. planifolia* in terms of overall vitality, whereas the latter as well as the hybrid had the highest growth rates. The performance of each *Vanilla* species, however, depended on land use system and light intensity, enabling us to identify species-specific niche preferences. Furthermore, soil bacterial diversity and specific bacterial phyla positively affected vanilla plant performance. We also observed significant effects of certain chemical soil variables (e.g. organic matter, pH) as well as functional tutor tree traits; indicating the important ecological interactions between vanilla orchids and their environment. Finally, the quantified aromatic potential of the vanilla pods demonstrated possibilities for market diversification, with *V. odorata* and the hybrid highly resembling the crop species in terms of aromatic profiling. Our results provide initial insights towards the integration of *Vanilla* CWRs within the proposed SPASHA approach. Additional research is required to further evaluate the feasibility (e.g. production, revenues, market regulations) of the proposed systems involving CWR use within the vanilla sector.

5.2 INTRODUCTION

Crop domestication, defined as the selection of valuable agronomic traits from crop species, has played a central role in modern civilization (Gepts, 2004; Chen et al., 2021). This selective process, however, greatly narrowed the genetic base of cultivated germplasm, known as the "domestication bottleneck" (Tanksley and McCouch, 1997; Meyer & Purugganan, 2013), and resulted in higher susceptibilities towards pests or diseases and a lower resilience to environmental changes (Ross-Ibarra et al., 2007; van Heerwaarden et al., 2011). A reduced genetic diversity has been observed in most modern crop species, and is exacerbated by the demand for high productivity and uniformity (Dempewolf et al., 2017). Furthermore, many crops were introduced in areas outside their centre of origin. During this so-called "dispersal bottleneck" (Zeder et al., 2006), only part of a crop's diversity was brought over, confining the crops' genetic base in these areas of introduction. A fundamental aspect in the history of crop domestication is that all crop species originated from their wild ancestors, known as crop wild relatives (CWRs). The genetic relationship between domesticated crops and their CWRs occurs at different levels. The primary gene pool usually includes the wild populations of the species from which the crop was once domesticated. Secondary and tertiary gene pools refer to closely related species, whereby gene transfer to the cultivated germplasm is possible through conventional breeding or more advanced engineering techniques (Meilleur and Hodgkin 2004; Maxted et al. 2006).

CWRs have a long evolutionary history across diverse environments (Maxted et al., 2015; Redden & Yadav, 2015). Consequently, they possess traits that might prove useful for crop improvement (Gur et al, 2004; Maxted et al. 2007; Takeda and Matsuoka 2008; Vincent et al., 2013; Dempewolf et al., 2017). Recently, and mainly due to the rising problems within modern food production systems, an increasing amount of attention has been paid to the use of CWRs for crop breeding, food security, and the development of resilient food production systems (Hajjar & Hodgkin, 2007; Prescott-Allen & Prescott-Allen, 2013; Yumurtaci, 2015; Goetsch et al., 2021). Substantial research has been carried out on the mayor global food crops, including rice, potato, maize, wheat, and soy bean, to name just a few. Vanilla, a high-value spice used in various industries, also passed through abovementioned domestication bottlenecks and associated production problems (Besse et al., 2004; Schluter et al., 2007; Bory et al., 2008; Divakaran et al., 2008; Verma et al., 2009), yet, the potential of its CWRs so far remains under-explored (Flanagan & Mosquera-Espinosa, 2016; Bramel & Frey, 2021). More surprisingly, the majority of incentives trying to counteract the observed production problems took place outside the centre of origin of the vanilla crop (Koyyappurath et al., 2015; Grisoni & Nany, 2021). Emphasis has been laid on

the primary CWR gene pool. The secondary or tertiary gene pools have received meagre attention. They may, however, possess a broad spectrum of traits useful to overcome present agronomic challenges as well as to diversify the vanilla market (Flanagan & Mosquera-Espinosa, 2016; Perez Silva et al., 2021). Recent studies demonstrated the high genetic diversity among *Vanilla* species, even within the crop species *Vanilla planifolia* Jacks. ex Andrews (e.g. Hu et al., 2019; Hasing et al., 2020; Chambers et al., 2021), indicating potential for improvement of flavour and production.

The Neotropics host, besides *V. planifolia*, at least 37 fragrant *Vanilla* species (Karremans et al., 2020). These CWRs are naturally growing in a variety of ecosystems, from old-growth forests and ridges to secondary forests and wetlands (Soto Arenas & Dressler, 2010). Hence, each species may require specific environmental conditions, such as light intensity, temperature and precipitation seasonality, and soil physicochemical characteristics. Furthermore, vanilla plants are known to depend on certain microbiota for optimal seed germination and growth (Porras-Alfaro, 2007; Johnson et al., 2021), interactions that may be species-specific as well. Traditional cultivation methods integrate the vanilla crop in secondary forests that resemble their natural habitats, or in intercropping agroforestry systems (Bory et al., 2008). The latter intents to mimic the essential environmental conditions vanilla plants depend on, and to create a buffer against abiotic and biotic stresses as well as market fluctuations (i.e. through the provision of a variety of crops). Nowadays, however, most commercial systems implement vanilla monocultures, with the latter ranging from plantations using tutor trees providing shade and vanilla vine support, to greenhouses using shade cloths (50% luminosity) and artificial poles (Havkin-Frenkel & Boulanger, 2018). These intense systems undoubtedly lead to higher production, yet, require more input as to mimic certain of the abovementioned plant-environment interactions. Moreover, monocultures have been associated with increased disease susceptibility of the crop species *V. planifolia* (Flanagan & Mosquera-Espinoza, 2016), whereby a rapid spread of the disease has led to entire crop losses.

From the above, and considering future threats related to climate change, we emphasize on the need for innovation at both crop species and system level, and propose a strategy that integrates a variety of *Vanilla* species with interesting organoleptic features, and this within resilient production systems. Due to the vulnerable status of most *Vanilla* CWRs (Bramel & Fey, 2021; Goettssch et al., 2021), a combined approach of *Vanilla* conservation and cultivation is key. Herewith, the previously defined joint land sparing/sharing approach (SPASHA) (Watteyn et al., 2020) has been proposed as a suitable strategy within our study region in southwest Costa Rica

– the Área de Conservación Osa (ACOSA). Considering the landscape context of ACOSA and following the SPASHA approach, cultivation systems could be implemented on both fallow and existing agricultural lands. Fallow or degraded lands could be converted to reforestation areas including vanilla as a high-value cash crop. Agricultural lands, dominantly oil palm monocultures, cacao plantations and pastures, could be transformed to diverse vanilla agroforestry systems. Yet, in order to provide practical recommendations, the performance of *Vanilla* CWRs in each of the abovementioned land use systems needs to be evaluated. The present study therefore aimed at providing initial insights into the cultivation potential of four *Vanilla* CWRs: *Vanilla hartii* Rolfe, *V. odorata* C. Presl, *V. pompona* Schiede, and *V. trigonocarpa* Hoehne, and this in naturally regenerated secondary forests and traditional cacao plantations. Over a period of two years, we examined the survival, vitality, growth and flowering patterns of these CWRs with the crop species *V. planifolia* and a disease-resistant hybrid ((*V. planifolia* x *V. pompona*) x *V. planifolia*). The obtained results will allow us to better understand CWR's cultivation potential so as to endorse their integration within the proposed regional production context referred to as SPASHA. Furthermore, we examined the aromatic profiles among the aforementioned species to further assess and compare their market potential.

5.3 MATERIALS AND METHODS

5.3.1 Study area and species

The study took place in the Área de Conservación Osa (ACOSA), a biodiversity hotspot in southwest Costa Rica hosting seven fragrant *Vanilla* species (Karremans et al., 2020; Watteyn et al., 2020). ACOSA is one of the most biodiverse places on earth relative to its size, and it is therefore seen as a conservation priority area (Kohlmann et al., 2010). The region consists of two national parks (Corcovado and Piedras Blancas) connected by a forest reserve (Golfo Dulce Forest Reserve), and surrounded by a fragmented landscape of villages and agricultural land. The biological corridor Osa serves as a buffer zone around abovementioned protected areas (SINAC, 2018). Annual precipitation ranges from 2,500 to 6,000 mm, with a rainy season from May to November, and the average temperature is 25 °C (Holdridge, 1967; Kappelle et al., 2003).

We studied the performance of six *Vanilla* species, of which two are commercially used species in Costa Rica: *Vanilla planifolia* and a disease-resistant hybrid ((*V. planifolia* x *V. pompona*) x *V. planifolia*). The other four species are fragrant *Vanilla* CWRs: *Vanilla hartii*, *V. odorata*, *V. pompona*, and *V. trigonocarpa*. Likewise *V. planifolia*, they belong to *Vanilla* sect. *Xanata*, and

can be seen as secondary gene pool CWRs as previous hybridizations demonstrated the possibility of gene transfer (e.g. *V. planifolia* x *V. pompona*). All are native to the lowland Neotropical rainforests, but with varying distributions (Watteyn et al., 2020). The species can be differentiated based on their leaves and stems, yet, flowers and fruits are usually required for species confirmation (Soto Arenas & Dressler, 2010; Karremans et al., 2020).

5.3.2 Experimental design

The experimental design consisted of 12 sites, of which six were located within naturally regenerated secondary forests (SF) and six in traditional cacao (*Theobroma cacao*) plantations (CP). CP were between four and six years old, characterized by a traditional 3 x 3 m cacao planting design and the presence of shade trees, with the latter also providing additional food commodities or ecosystem services (e.g. *Inga* spp., *Gliricidia sepium*). The twelve sites were spread across ACOSA, and were selected based on farmers' willingness to cooperate with this study, and the equal spread of CP and SF sites across a range of elevations (Fig. 5.1).

In each of the sites, six individuals per *Vanilla* species were planted in June 2019, totalling 36 plants per site. One week before planting, vegetative cuttings of the hybrid and *V. planifolia* were collected from local plantations, and *Vanilla* CWRs cuttings from wild populations present in ACOSA. Cuttings were sealed to avoid potential proliferation of fungal and bacterial contamination during transportation from collection to experimental site. Each site consisted of six rows, in which each row contained one individual of each species. The planting order of the different *Vanilla* species varied among the rows to avoid potential edge-effects (Fig. 5.2).

Vanilla being a climbing orchid, needs a so-called tutor tree. Following the traditional method, 1.60 m long branch cuttings of *Erythrina* were planted as nitrogen-fixing tutor trees between the cacao rows in the CP, with a distance of 1.5 m between cacao and vanilla tutor tree, and 3 m between the tutor trees of the same row. The already available shade in the CP sites allowed us to directly integrate the vanilla cuttings after tutor tree planting, whereas tutor trees are usually planted one year ahead if implemented on a cleared area. Within the SF, we selected trees that had a diameter at breast height (DBH) ≥ 5 cm and allowed us to roughly generate rows. The eight-nodded cuttings were planted by placing the lower three nodes onto the soil and tying the upper five nodes onto the tutor tree using biodegradable jute twine to avoid strangulation. The part that was placed on the soil, was covered with onsite available organic material.

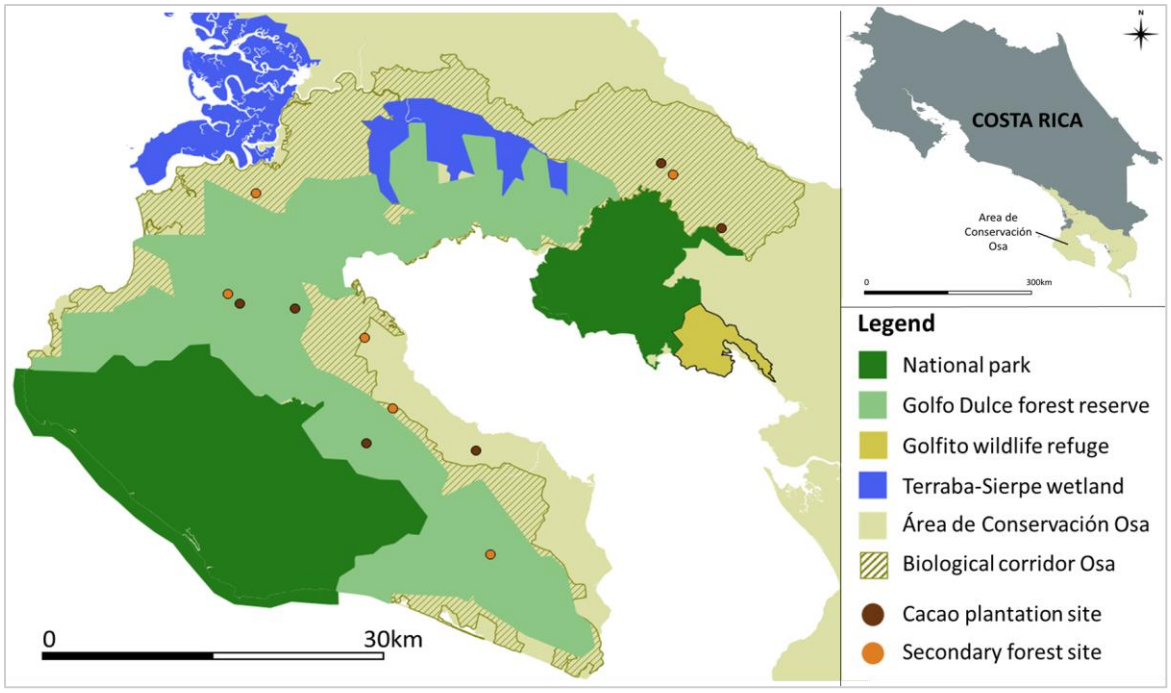

Fig. 5.1. *The location of the twelve experimental sites, randomly spread across the study area ACOSA. The orange and red dots represent the sites in secondary forests and cacao plantations, respectively.*

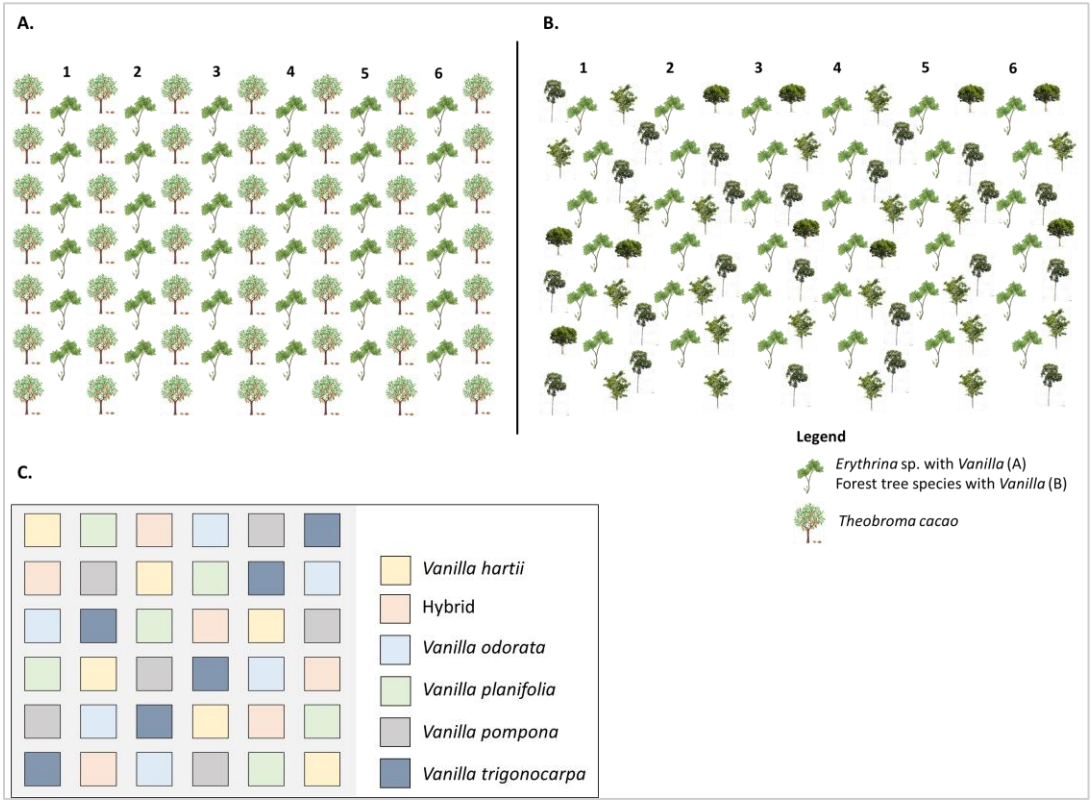

Fig. 5.2. *Planting design in traditional cacao plantations (3 m x 3 m) (A), and naturally regenerated secondary forests (B). The numbers (1-6) refer to the six rows per site, in which each row contains one individual of each Vanilla species, following a random planting design across the site (C).*

5.3.3 Data collection

Vanilla plant performance

For two consecutive years, we collected data related to the performance of the vanilla plants. More specifically, we recorded plant survival and measured variables related to plant vitality, growth and flowering (Table A4.1) during three periods, starting six months after planting: December 2019, June 2020 and December 2020. We selected these specific moments as they correspond with the dry (December) and rainy (June) season. First, the survival of each plant was documented (dead or alive). A side note here is that, if we encountered the planted cutting in a very bad condition (e.g. nearly dead), but a new shoot was observed, the individual was marked as alive. Next, we measured the vitality of the planted cutting and the newly developed shoot(s), by examining (a) herbivore damage, (b) presence of pathogenic fungi and bacteria, (c) leaf discoloration, (d) number of missing leaves, and (e) number of missing shoot apices. The variables herbivory, pathogens and leaf discoloration were scored as followed: insignificant, moderate, or strong (Fig. A4.1). The percentage of missing leaves was calculated by dividing the number of missing leaves by the number of internodes, as each internode normally bears one leaf. The percentage of missing shoot apices was calculated by dividing the number of missing shoot apices by the number of new shoots.

The growth of each vanilla plant was quantified via (a) the presence or absence of roots, (b) the presence and number of new shoots, (c) the total number of internodes per shoot, (d) the length of each internode, (e) the diameter of each internode, and (f) the leaf area of six average-sized leaves. The following variables were derived from the above: shoot length (sum of internode lengths per shoot), total vine length (sum of internode lengths of all shoots), mean internode length and diameter, mean and total leaf area. During the first measurement period, we removed three medium-sized leaves from each vanilla plant and measured their dimensions. The leaf area was calculated using the ImageJ 1.53n (Schneider et al., 2012). For each *Vanilla* species, a linear regression model predicted leaf area with the variables leaf length and width, allowing us to continue with non-destructive leaf area measurements. Considering vanilla plants generally start to flower three to four years after planting the vegetative cutting (Havkin-Frankel and Belanger, 2018), we did not expect to observe any flowering within the timeframe of our study. However, flowers were detected at the end of 2020 (last monitoring period) and throughout 2021 for the species *V. hartii*, *V. odorata*, and *V. pompona*, of which the number of inflorescences and flowers were recorded. Furthermore, the number of flowering plants per species was recorded to calculate flowering probability (%) less than two years after planting.

Environmental variables

Besides the explanatory variables of interest, i.e. *Vanilla* species and land use system, we measured several environmental variables to better understand their potential effect on individual vanilla plant performance.

The following variables were measured at site level: (a) elevation (m), (b) slope (%), (c) tree species diversity, by identifying and counting all trees with a DBH ≥ 5 cm, (d) soil bacterial diversity, by 16S ribosomal RNA (rRNA) sequencing and amplicon sequence variant (ASV) inference, and (e) soil chemical properties such as pH, acidity, macronutrients (P, K, Ca, Mg), micronutrients (Fe, Zn, Cu, Mn) and organic matter. Twenty-five random bulk soil samples (0-10 cm depth) were collected from each site in 2019 and 2020. A composite soil sample was obtained by mixing the 25 samples per site. Large woody litter was removed from the soil samples, and the soil was air-dried and sieved to be stored at 4°C for analysis of abovementioned soil chemical properties, and at -20°C for metabarcoding analysis of soil microbiota. DNA was extracted from 250 mg of soil sample, in triplicate, using a DNeasy PowerSoil Kit (Qiagen) and the MM400 homogenizer (Retsch GmbH, Haan, Germany), following the manufacturer's guidelines. The V4 region of bacterial 16S rRNA gene was amplified using the barcoded primers F515 and R806, as described previously (Caporaso et al., 2011). Libraries were sequenced on the Illumina NovaSeq 6000 using 2 x 250 bp paired reads, with 100,000 reads per sample (Novogene Corporation Inc., Sacramento, CA).

The variables measured at vanilla plant level were (a) relative photosynthetic active radiation (PAR), i.e. the portion of the light spectrum used by plants for photosynthesis (400-700 nm), using a LI-190R quantum sensor held at a height of approximately 2 m alongside each vanilla plant and a LI-1500 light sensor logger placed in an adjacent open field as reference, (b) tutor tree species, (c) tutor tree DBH, and (d) tutor tree height.

Additionally, to explain potential effects of the tutor tree species and on-site tree species diversity on vanilla plant performance, we collected functional trait data from the TRY (Kattge et al., 2020) and BIEN (Maitner et al., 2017) databases. For each tree species, we gathered information on the following available traits, which relate to nutrient cycling and nutritional quality, and thus plant performance (Lavorel & Grigulis et al., 2012; Díaz et al., 2016): leaf carbon (C), leaf nitrogen (N), leaf phosphorous (P), specific leaf area (SLA), leaf dry matter content (LDMC), leaf thickness, lamina fracture toughness, leaf C:N ratio, leaf C:P ratio, leaf N:P ratio, and leaf lignin.

Aromatic potential of vanilla pods

We collected two pods per *Vanilla* species for aromatic compound analysis. Pods of the hybrid were harvested from the same vanilla plantation that provided the vegetative cuttings, whereas pods of the *Vanilla* CWRs were harvested from the same wild populations from where we collected the cuttings (Table A4.2). No pods of *V. planifolia* could be harvested due to the absence of *V. planifolia* plantations within our study area. As such, we used *V. planifolia* pods available at Instituto Tecnológico de Tuxtepec, where subsequent analysis was carried out, and also compared with data available from the literature (e.g. Pérez Silva et al., 2021) to compare the aromatic potential among all species. All pods were harvested just before complete maturation, i.e. eight to nine months after pollination, as the aromatic compounds are mainly developed during the last months of fruit growth, and are accumulated as glycosides (Palama et al., 2009). Pods were freeze-dried to minimize possible enzymatic degradation, and stored at -20 °C until analysis. The methodology described in Pérez-Silva et al. (2021) was applied to analyse the aromatic compounds via high-performance liquid chromatography (HPLC) analysis, using an Agilent 1100 series apparatus equipped with a UV-VIS detector (G1314A), degasser (G1379A), column oven (G1316A), quaternary pump unit (G1311A), and autosampler (G1313A) controlled by OpenLAB CDS EZChrom Edition software (Agilent Technologies Inc., 2013). The following reference compounds were included: glucovanillin, vanillin, vanillic acid, vanillyl alcohol, 4-hydroxybenzaldehyde (pHB), pHB acid, pHB alcohol, anisyl alcohol, and anisyl acid (Sigma-Aldrich, Chromadex). A Zorbax Eclipse column plus XDB C18 (150 mm x 4.6 mm; 5 µm, Agilent Technologies, Mexico) was used and the mobile phase involved a mixture of the solvents 10–2 M H_3 PO_4 and HPLC-grade methanol. Solutions of the compounds (1 to 100 mg/l) were separately prepared in the mobile phase and injected into the HPLC system to build the calibration curve.

5.3.4 Data analysis

Data exploration on Vanilla plant performance

A factorial analysis of mixed data (FAMD) (FactoMineR – Lê et al., 2008; FactoExtra – Kassambara & Mundt, 2017) was used to reduce the complexity of the dataset encompassing the measured plant vitality and growth traits. This method can be applied to datasets containing both continuous and categorical variables, whereby the continuous variables are scaled to unit variance and the categorical variables are transformed into a disjunctive data table using multiple correspondence analysis (MCA) scaling (Pages, 2004). We examined the correlation between the measured traits and analysed their contribution to the observed variation within the dataset, and subsequently grouped the individuals by *Vanilla* species, land use system, site and

measurement period to explore initial patterns. Next, we applied a principal component analysis (PCA) with scaled variables (Legendre & Gallagher, 2001) to examine potential correlations among the 12 continuous soil chemical variables measured at site level, and as such reduce the dimensionality of this dataset.

Regarding the identified soil bacteria, primer and adapter sequences were removed with Cutadapt (Martin, 2011), ASVs were inferred with DADA2 (Callahan et al., 2016), taxonomic assignment was performed with a naive Bayesian classifier (Wang et al. 2007) and the SILVA SSU r138.1 database (Quast et al., 2012). For alpha diversity analysis, inferred ASVs were pre-processed by removing those classified as "Chloroplast", "Mitochondria", or "Eukaryota", by filtering out those not assigned to Bacteria or Archaea, and then filtered raw counts were normalized by scaling with ranked subsampling (Beule & Karlovsky, 2020). Alpha diversity metrics were calculated to compare the soil bacterial diversity among site and land use system (phyloseq – McMurdie & Holmes, 2013; vegan – Oksanen et al., 2020), using Kruskal-Wallis test and Dunn's post-hoc tests with multiple comparison correction via the Benjamini-Hochberg adjustment to control the false discovery rate and Mann-Whitney test. Bacterial phyla of which their relative abundances significantly differed among sites were also identified via an ANOVA-like differential abundance analysis of compositional data on filtered raw counts (ALDEx2 – Fernandes et al., 2013). The tree species diversity was compared among land use system by calculating and comparing the same alpha diversity metrics as mentioned above. The community-weighted means (CWM) of each of the functional tree traits as well as the functional tree diversity were calculated at site level (FD – Laliberté et al., 2014).

Vanilla plant performance models

We developed (generalized) linear mixed models (lme4 package) (Bates et al., 2014) to test the effects of the fixed variables *Vanilla* species, land use system and measured environmental variables on vanilla plant survival, vitality and growth, and to account for the correlation structure in our data by including the random effect of site nested within period of measurement (Pinheiro & Bates, 2000). In case of non-significant random effects, i.e. no significant difference in Akaike's information criterion (AIC) between models with or without the random effect, logistic or linear models were used. We visually examined residual plots to ensure that the assumptions of linearity and homoscedasticity were met (Zuur et al., 2006; Zuur & Ieno, 2016). Variance inflation factors (VIF) were checked to ensure absence of multicollinearity among the predictor variables, whereby VIFs greater than 10 suggest strong collinearity (Shrestha, 2020). Due to the large number of potential predictor variables, we first obtained minimal adequate models using the buildmer and

drop1 functions (Bates et al., 2014; Voeten, 2020). As such, significant main effects were identified and subsequent graphical data exploration suggested possible interactions to be tested. The AIC and restricted maximum likelihood (REML) were used to choose the best models. Standardization was obtained by fitting the models on a standardized dataset.

Aromatic potential of vanilla pods

We carried out a PCA to visually explore the differences and similarities among the *Vanilla* species, based on the aromatic profiles derived from HPLC quantifications. Furthermore, the values of the quantified aromatic compounds were averaged per compound and compared among the *Vanilla* species.

All statistical analyses were performed using R version 4.1.1.

5.4 RESULTS

5.4.1 Exploration of *Vanilla* plant performance

The first and second FAMD axes explained, respectively, 34.1% and 14.1 % of the variation within the plant trait dataset (Fig. A4.2). The variables with the largest contribution to the first axis were associated with plant growth: number of leaves, vine length, number of internodes and total leaf area. This axis separated *V. hartii*, *V. odorata* and *V. trigonocarpa* (left) from *V. planifolia*, *V. pompona* and the hybrid (right) (Fig. A4.3), and secondary forest (left) from cacao plantations (right) (Fig. A4.4). The main contributing variables to the second axis were related to plant vitality, such as number of missing shoot apices, percentage of missing leaves, number of new shoots, presence of herbivory or pathogens, mean internode diameter, and mean leaf area. This axis separated *V. hartii*, *V. odorata* and *V. planifolia* (top) from *V. pompona* and *V. hybrid* (bottom). The positive correlation between herbivore leaf damage and pathogen presence can be explained by the fact that physical destruction makes plants more susceptible for pathogen attacks (Savatin et al., 2014). The negative correlation of mean internode diameter and mean leaf area with the other vitality variables indicates the role of vigorous vegetative material in plant robustness (Koyyappurath et al., 2015). Based on the FAMD, axis 1 and 2 were used as proxies for, respectively, the plant growth and vitality response variables in subsequent modelling. The PCA applied to the soil chemical variables explained 61.5 % of the observed variation within this dataset. Variables contributing to the first and second axis were associated to, respectively, acidity (pH, acidity, Fe, Ca, K) and nutrient cycling (e.g. Mg, organic matter, P) (Fig. A4.5), and were added as predictor variables soil pca1 and soil pca2 in subsequent modelling.

The dominant soil bacterial phyla were Proteobacteria (27.37 %), Acidobacteriota (22.04 %), Actinobacteriota (11.42%), Verrucomicrobiota (10.25 %), Planctomycetota (6.37 %), Myxococcota (3.62 %), Chloroflexi (3.46 %), Firmicutes (3.04 %), Bacteroidota (2.47 %), Methylomirabilota (1.88 %), Gemmatimonadota (1.73 %), Latescibacterota (1.23 %), Desulfobacterota (1.06 %), Nitrospirota (0.65 %), and Crenarchaeota (0.54 %) (Fig. A4.6). Significant differences were found among the sites in alpha-diversity measures (H = 29.53, P = 0.001) (Fig. A4.7) and the relative abundances of the following bacterial phyla (Table A4.3): Acidobacteriota, Actinobacteriota, Armatimonadota, Bacteroidota, Calditrichota, Chloroflexi, Crenarchaeota, Dependentiae, Desulfobacterota, Firmicutes, Gemmatimonadota, Latescibacterota, Methylomirabilota, Myxococcota, Nitrospirota, Planctomycetota, Proteobacteria, and Verrucomicrobiota. Hence, bacterial alpha-diversity (e.g. Shannon-Wiener index) and the relative abundance of the abovementioned phyla were added as potential predictor variables in subsequent modelling. Tree alpha-diversity differed significantly among land use systems (W = 5, P = 0.045) (Fig. A4.8), and we observed differences in tree species composition among sites and land use systems (ANOSIM = 0.92, p = 0.002) (Fig. A4.9). The CMWs of the functional tree traits and functional tree diversity were added as predictor variables in subsequent modelling, along with the trait values of the tutor trees.

5.4.2 Vanilla plant performance models

Plant survival

We fitted a logistic model as the random effect did not prove significant. The final model (AIC = 414.27) predicted plant survival with *Vanilla* species, land use system, light, soil bacterial diversity, soil pca2, and the relative abundance of the bacterial phyla Firmicutes and Verrucomicrobiota (Table 5.1). Survival probability was higher in SF compared to CP, and although there was no significant interaction term between *Vanilla* species and land use system, we observed a higher survival of *V. hartii* and *V. trigonocarpa* in SF compared to CP, while *V. planifolia* had a higher survival rate in CP (Fig. 5.3-A). The other species had similar survival probabilities in both systems. There was a significant interaction between *Vanilla* species and light (Fig. 5.4-A). Light availability had a positive effect on the survival of the hybrid, *V. planifolia*, *V. pompona*, and *V. trigonocarpa*, while it negatively affected *V. hartii* and had no effect on *V. odorata*. Bacterial diversity, and the relative abundance of the bacterial phyla Firmicutes and Verrucomicrobiota positively affected plant survival probabilities (Fig. 5.5-A, Fig. 5.6-A,B).

Plant vitality

Plant vitality was measured via (a) the condition of the planted vegetative cuttings, determined by the visual assessment of pathogen presence (hereafter vitality_1), and (b) the vitality of the newly developed shoots, i.e. the coordinates of the second FAMD axis (hereafter vitality_2) (see 3.1.1.). We fitted a logistic mixed vitality_1 model as the random effect proved significant. The final model (AIC = 876.49) predicted vitality_1 with *Vanilla* species, land use system, light, soil bacterial diversity, and the bacterial phylum Firmicutes (Table 5.2). Overall, the vitality_1 of the hybrid and the CWRs was higher compared to the crop species *V. planifolia*, but *Vanilla* species significantly interacted with both land use system and light (Fig. 5.3-B, Fig. 5.4-B). The soil bacterial diversity and the relative abundance of the bacterial phylum Firmicutes positively affected vitality_1 (Fig. 5.5-B, Fig. 5.6-C). Next, we fitted a linear mixed effect model for vitality_2. The final model (AIC = 2975.55) predicted vitality_2 with *Vanilla* species, land use system, the vitality of the planted vegetative cutting, and soil pca1 (Table 5.3). There was a significant interaction between *Vanilla* species and land use system. The vitality_2 of the hybrid and *V. pompona* was higher compared to the other species, in both CP and SF (Fig. 5.3-C). *Vanilla planifolia* had a lower vitality_2 in SF compared to CP, whereas the other species performed similar in both systems. The vitality of the planted vegetative cutting (vitality_1) significantly affected the vitality of the newly developed shoots, whereby a low vitality_1 led to a lower vitality_2 (Fig. 5.7-A). Soil pca1 negatively affected plant vitality (Fig. 5.7-B).

Plant growth

Lastly, we fitted a linear mixed model for vanilla plant growth. The final model (AIC = 3799.84) predicted the growth (i.e. the coordinates of the first FAMD axis) of the new shoots with *Vanilla* species, land use system, soil pca2, SLA and leaf C:N ratio of tutor trees, soil bacterial diversity, and the bacterial phyla Acidobacteriota, Bacteroidota, and Firmicutes (Table 5.4). The variable *Vanilla* species had a significant interaction with both land use systems and light (Fig. 5.3-D). The hybrid, *V. odorata*, and *V. planifolia* grew better in CP compared to SF, while the growth of the other three species was similar in both systems. Furthermore, the hybrid and *V. planifolia* grew better with increasing light, while light did not seem to affect the growth of the other species (Fig. 5.4-C). Soil bacterial diversity as well as the abundance of the bacterial phyla Acidobacteriota, Bacteroidetes and Firmicutes positively affect plant growth (Fig. 5.5-C, Fig. 5.6-D,E,F). Finally, we observe a positive effect of soil pca2 and the SLA of the tutor trees on plant growth, whereas the leaf C:N ratio of the tutor trees negatively affected plant growth (Fig. 5.7-C,D,E). Both vitality and growth varied among sites, indicating potential effects of inter-site variability that were not captured by the fixed variables added to the models.

Table 5.1. *Model estimates of the logistic plant survival model. SE = standard error. CI = confidence interval.*

Fixed effect	**Coefficient**		**SE**	**Odds Ratio**	**95% CI**	
Intercept	-16.31	**	6.19	0.00	0.00	0.01
Land use system (ref. CP)						
SF	2.60	***	0.47	13.45	5.48	34.96
***Vanilla* species : Light**						
Hybrid : Light	0.76	*	0.37	2.14	1.22	5.26
V. odorata : Light	0.20		0.14	1.22	1.00	1.76
V. planifolia : Light	0.69	**	0.21	1.99	1.43	3.19
V. pompona : Light	0.35	*	0.18	1.42	1.10	2.15
V. trigonocarpa : Light	0.07	*	0.03	1.08	1.01	1.15
***Vanilla* species** (ref. *V. hartii*)						
Hybrid	-1.94	*	0.97	0.14	0.02	0.98
V. odorata	-0.27		0.93	0.76	0.13	4.96
V. planifolia	-3.36	***	0.74	0.03	0.01	0.14
V. pompona	-1.57		0.83	0.21	0.04	1.03
V. trigonocarpa	-1.89	**	0.61	0.15	0.04	0.47
Light	-0.02		0.47	1.00	0.96	1.04
Soil bacterial diversity	1.65	*	0.84	5.23	1.01	28.01
Soil pca2	0.33	***	0.10	1.39	1.15	1.69
Firmicutes	0.78	***	0.16	2.17	1.62	3.06
Verrucomicrobiota	0.38	***	0.08	1.46	1.25	1.73
AIC	414.27					
Log likelihood	-190.13					

Significante shown at *** ≤ 0.001, ** ≤ 0.01, * ≤ 0.05

Table 5.2. *Model estimates of the logistic mixed effect vitality_1 model. SE = standard error. CI = confidence interval.*

Fixed effect	**Coefficient**		**SE**	**Odds Ratio**	**95% CI**	
Intercept	-6.28	*	3.33	0.00	0.00	1.33
***Vanilla* species : Land use system**						
Hybrid : SF	2.86	**	1.05	17.47	2.42	15.75
V. odorata : SF	-0.41		0.80	0.67	0.14	3.20
V. planifolia : SF	-1.53	*	0.71	0.22	0.05	0.88
V. pompona : SF	0.01		0.81	1.00	0.20	4.95
V. trigonocarpa : SF	0.74		0.73	2.09	0.50	8.95
***Vanilla* species : Light**						
Hybrid : Light	0.65	**	0.24	1.91	1.31	3.36
V. odorata : Light	0.10		0.06	1.11	1.01	1.26
V. planifolia : Light	0.04		0.02	1.04	0.99	1.09
V. pompona : Light	0.06		0.03	1.07	1.00	1.15
V. trigonocarpa : Light	0.05		0.03	1.05	0.99	1.11
***Vanilla* species** (ref. *V. hartii*)						
Hybrid	-3.59	**	1.10	0.03	0.00	0.21
V. odorata	-0.57		0.71	0.56	0.14	2.31
V. planifolia	-2.21	***	0.59	0.11	0.03	0.33
V. pompona	-0.90		0.72	0.41	0.10	1.67
V. trigonocarpa	-1.69	**	0.61	0.18	0.05	0.59
Land use system (ref. CP)						
SF	0.27		0.59	1.31	0.40	4.19
Light	-0.01		0.02	0.99	0.95	1.03
Soil bacterial diversity	1.10	*	0.43	2.94	1.26	6.79
Firmicutes	0.10	*	0.05	1.10	0.99	1.23
Random effect	**Variance**		**St. dev.**			
σ2	3.29		1.93			
τ00 Site:Period	0.19		0.33			
τ00 Period	1.28		1.13			
AIC	876.49					
Log likelihood	-416.20					

Significance shown at *** ≤ 0.001, ** ≤ 0.01, * ≤ 0.05

Table 5.3. *Model estimates of the linear mixed effect vitality_2 model. CI = confidence interval.*

Fixed effect	**Coefficient**		**95% CI**	
Intercept	-0.04		-1.41	1.33
***Vanilla* species : Land use system**				
Hybrid: SF	-0.39		-0.90	0.12
V. odorata : SF	-0.20		-0.68	0.29
V. planifolia : SF	-0.52	**	-1.01	-0.02
V. pompona : SF	-0.28		-1.19	0.18
V. trigonocarpa : SF	-0.03		-0.54	0.48
***Vanilla* species** (ref. *V. hartii*)				
Hybrid	2.19	***	1.85	2.53
V. odorata	0.01		-0.32	0.35
V. planifolia	0.08		-0.26	0.43
V. pompona	2.02	***	1.66	2.38
V. trigonocarpa	0.47	*	0.11	0.84
Land use system (ref. CP)				
SF	0.16		-0.24	0.57
Vitality_1	0.79	***	0.56	1.02
Soil pca1	-0.12	**	-0.20	-0.04
Random effect	**Variance**		**St. dev.**	
σ2	1.22		1.11	
τ00 Site:Period	0.04		0.19	
τ00 Period	0.91		0.95	
AIC	2975.55			
Log Likelihood	-1468.78			

Significance shown at *** ≤ 0.001, ** ≤ 0.01, * ≤ 0.05

Table 5.4. *Model estimates of linear mixed effect growth model. CI = confidence interval.*

Fixed effects	**Coefficient**		**95% CI**	
Intercept	-35.44	***	-56.54	-14.35
***Vanilla* species : Land use system**				
Hybrid : SF	-0.96	**	-1.87	-0.05
V. odorata : SF	-1.17	**	-2.01	-0.33
V. planifolia : SF	-1.99	***	-2.86	-1.13
V. pompona : SF	-0.24		-1.25	0.77
V. trigonocarpa : SF	-0.34		-1.27	0.59
***Vanilla* species : Light**				
Hybrid : Light	0.12	***	0.08	0.15
V. odorata : Light	0.00		-0.03	0.04
V. planifolia : Light	0.08	***	0.04	0.11
V. pompona : Light	0.03		-0.01	0.06
V. trigonocarpa : Light	0.02		-0.02	0.06
***Vanilla* species** (ref. *V. hartii*)				
Hybrid	1.34	***	0.54	2.14
V. odorata	0.76	*	0.02	1.51
V. planifolia	1.68	***	0.92	2.44
V. pompona	0.74		-0.19	1.67
V. trigonocarpa	-0.81		-1.65	0.03
Land use system (ref. CP)				
SF	2.48	***	1.09	3.87
Light	-0.02		-0.04	0.01
Acidobacteriota	0.18	*	0.01	0.37
Bacteroidetes	0.85	***	0.35	1.35
Firmicutes	0.47	**	0.15	0.78
Soil pca2	0.41	***	0.19	0.64
Tree SLA	0.43	***	0.17	0.69
Tree leaf C:N ratio	-0.54	***	-0.80	-0.28
Random effect	**Variance**		**St. dev.**	
σ2	2.48		1.57	
τ00 Period:Site	0.39		0.62	
τ00 Period	1.26		1.12	
AIC	3799.84			
Log likelihood	-1869.92			

Significance shown at *** ≤ 0.001, ** ≤ 0.01, * ≤ 0.05

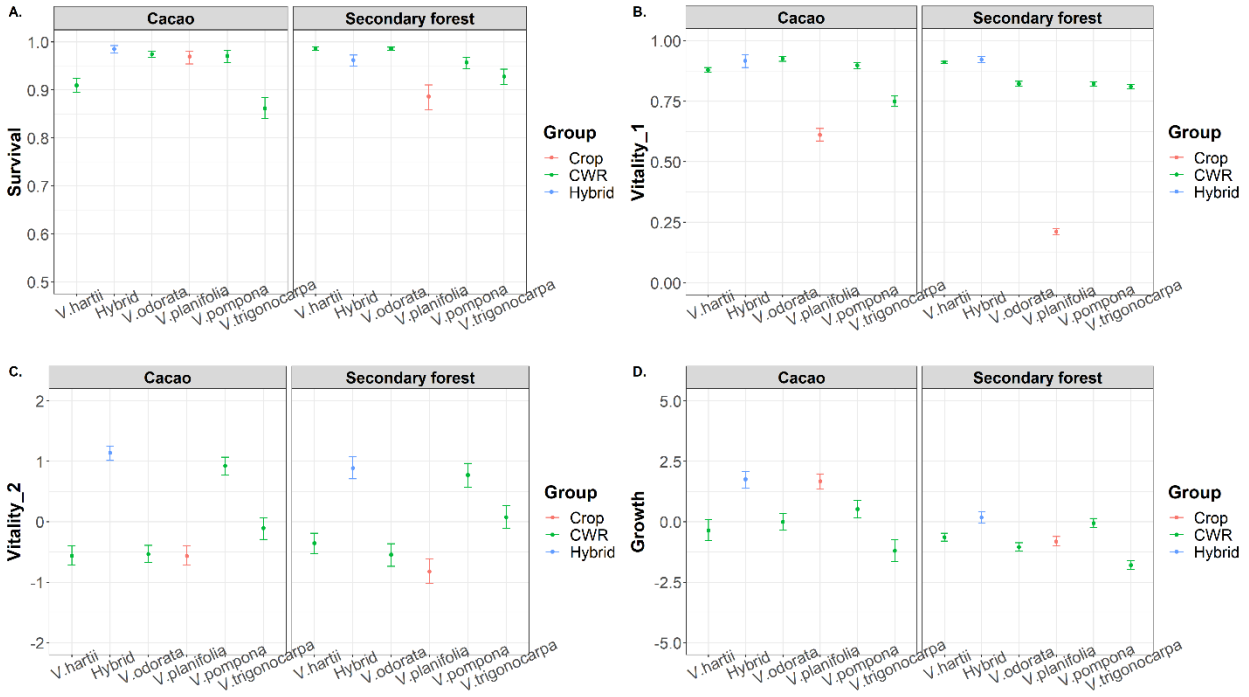

Fig. 5.3. *Predicted effect of land use system on vanilla plant survival (A), vitality of the planted cutting (B), vitality of the new shoots (C), and vanilla plant growth (D), given per Vanilla species and coloured according group (CWR, crop species, hybrid).*

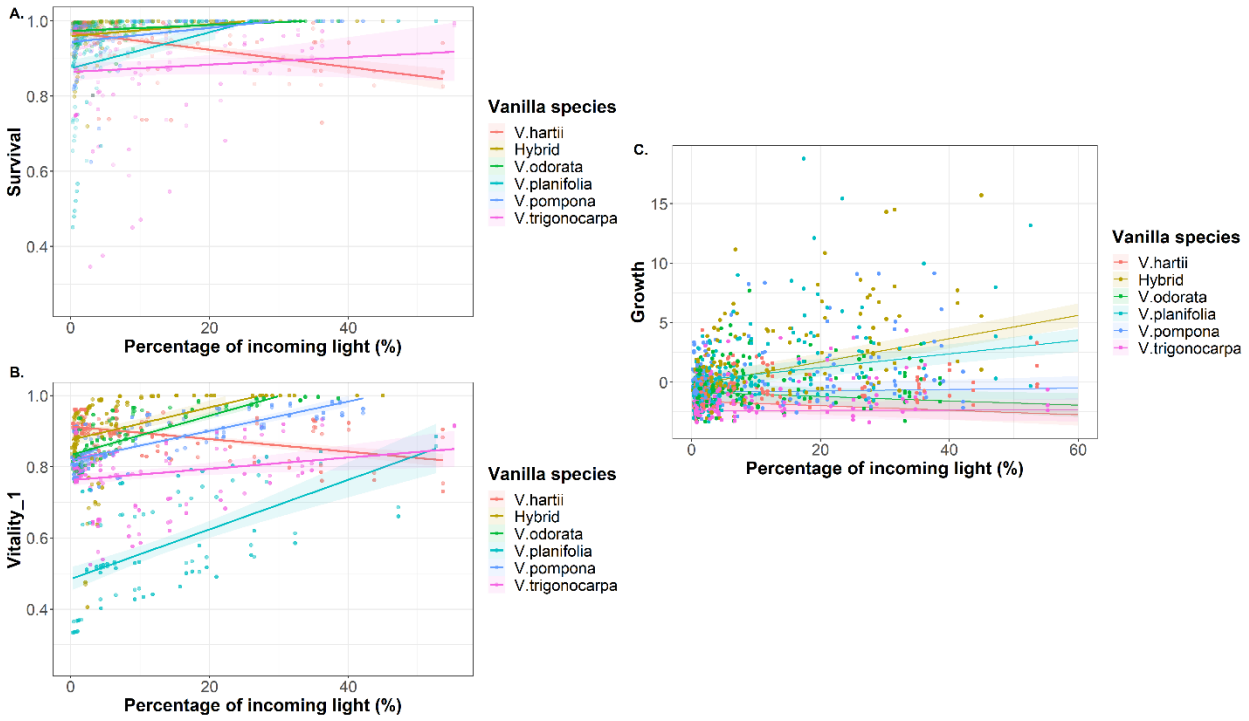

Fig. 5.4. *Predicted interaction effect of incoming light intensity (%) on vanilla plant survival (A), vitality of the planted cutting (B), vitality of the new shoots (C), and vanilla plant growth (D), coloured according to Vanilla species.*

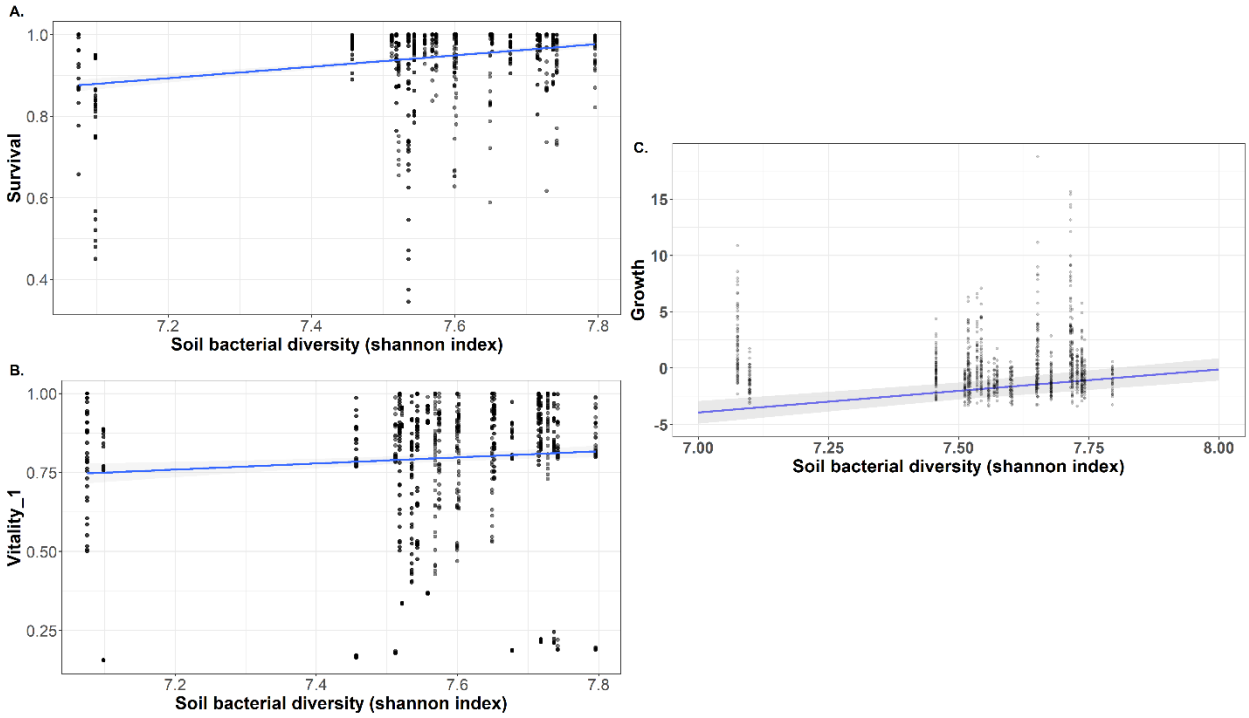

Fig. 5.5. *Predicted effect of soil bacterial diversity on vanilla plant survival (A), vitality of the planted cutting (B), and vanilla plant growth (C). The soil bacterial diversity is represented by the Shannon-Weiner species diversity index.*

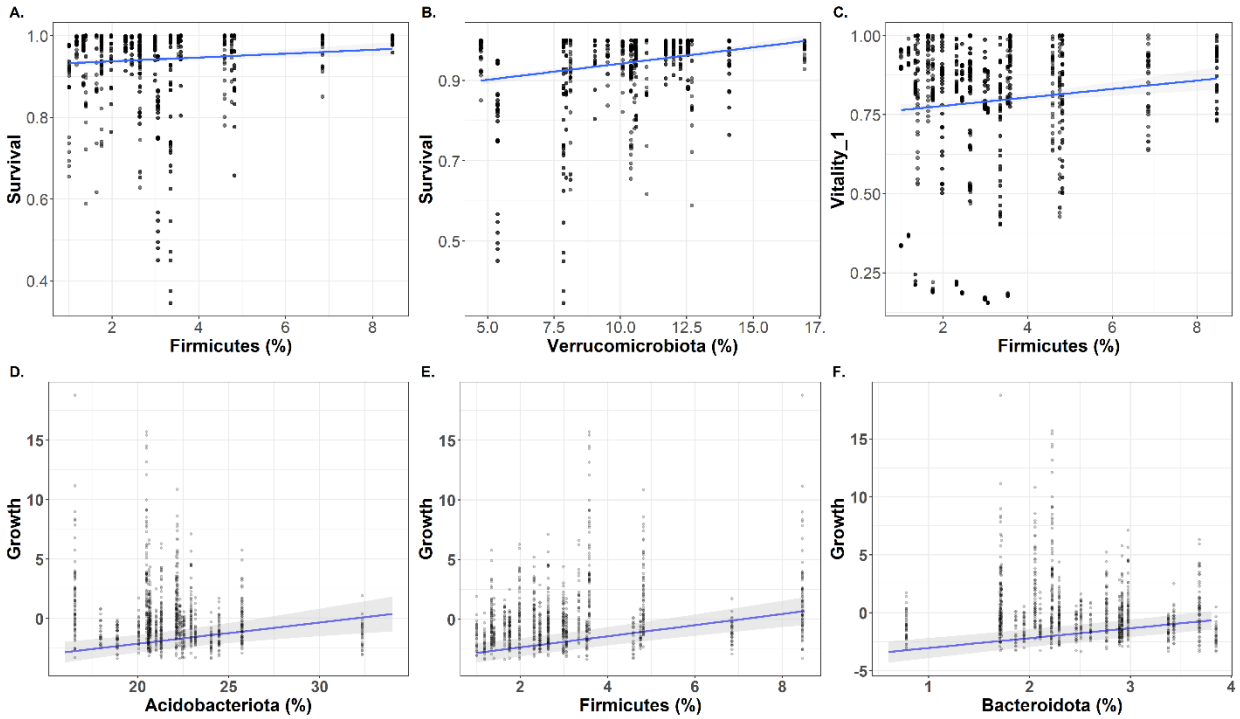

Fig. 5.6. *Predicted effect of the relative abundance (RA) of specific bacterial phyla on vanilla plant survival (A, B), vitality of the planted cutting (C), and vanilla plant growth (D, E, F). The RA of a phylum was calculated by dividing the number of ASVs of that phylum by the total number of ASVs in the sample.*

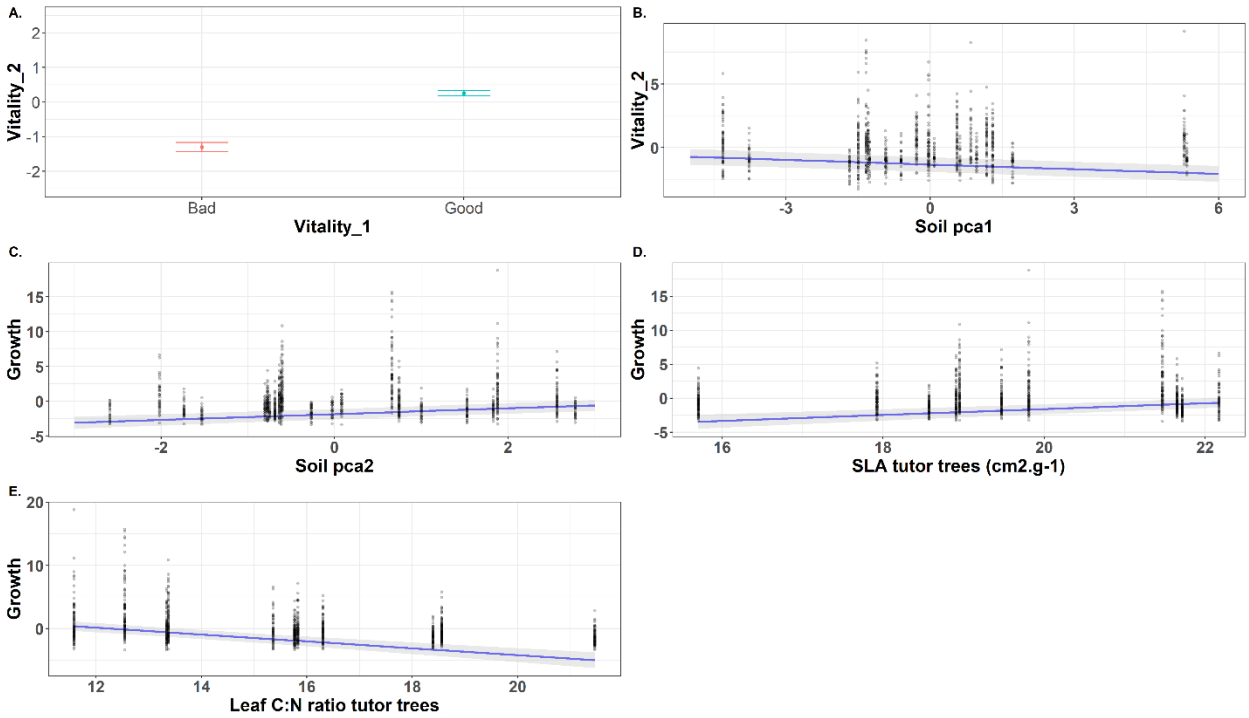

Fig. 5.7. *Predicted effect of the vitality of the planted cutting and soil pca1 on the vitliaty of new shoots (respectively A and B), and soil pca2, SLA of the tutor trees, and leaf C:N ratio on vanilla plant growth (respectively C, D and E).*

Plant production

The species *V. hartii*, *V. odorata*, and *V. pompona* started to produce flowers one and a half to two years after planting, i.e. December 2020 for *V. odorata* and January-March 2021 for *V. hartii* and *V. pompona*. The number of inflorescences and flowers per plant did not differ among species (Table 5.5). The hybrid, *V. planifolia* and *V. trigonocarpa* did not flower within the timeframe of this study.

Table 5.5. *Flower probability per Vanilla species after one and a half to two years of planting, demonstrating the average number of inflorescences and flowers produced per plant.*

***Vanilla* species**	**Flowering probability (%)**	**# inflorescences per plant (mean ± SD[1])**	**# flowers per plant (mean ± SD[1])**
Vanilla hartii	2.90	1.80 ± 0.84	12.00 ± 4.95
Vanilla odorata	4.84	2.00 ± 1.58	12.70 ± 10.00
Vanilla pompona	4.29	2.86 ± 2.12	20.70 16.40

[1]SD = Standard deviation.

5.4.3 Aromatic potential of vanilla pods

The aromatic compound analysis included five *Vanilla* CWRs (*V. hartii*, *V. helleri*, *V. odorata*, *V. pompona*, *V. trigonocarpa*) and the hybrid; all belonging to *Vanilla* section *Xanata*. For each species, the average contents of glucovanillin and aglycones released by hydrolysis were calculated, and the aglycones were grouped per family (vanillyl, p-hydroxybenzyl (pHB) and anisyl) (Table 5.6). The vanillin potential was determined by adding the content of free vanillin and 48.4% (i.e. ratio of vanillin/glucovanillin molecular weights) of the content of glucovanillin directly determined by HPLC (without enzymatic hydrolysis) – the same protocol as described in Pérez-Silva et al. (2021). The first and second PCA axis explained 64.3 % of the total variation within the dataset (Fig. 5.8). The first axis was correlated with vanillin potential, pHB acid, vanillic acid on the one hand, and anisic alcohol on the other hand. The second axis was correlated with pHB alcohol and total pHB. *Vanilla pompona* and *V. hartii* were clearly separated at the left end of the first axis, as the pHB acid content and vanillin potential were below the detection threshold, whereas the vanillin-rich species, *V. planifolia*, *V. odorata* and the hybrid, were found at the very right end. The high anisyl alcohol content in *V. hartii* and *V. pompona* further explains their left-sided position along the first axis. The lower vanillin potential and pHB acid content of *V. helleri* and *V. trigonocarpa* explains their more centric position. The second axis mainly separated *V. trigonocarpa*, *V. planifolia* and *V. pompona* due their higher pHB alcohol content, with the latter found in all species but in different quantities. *Vanilla odorata* and the hybrid contained all compounds except for anisyl acid. Apart from vanillic acid, *Vanilla helleri* contained all of the examined compounds, yet, all in rather small quantities.

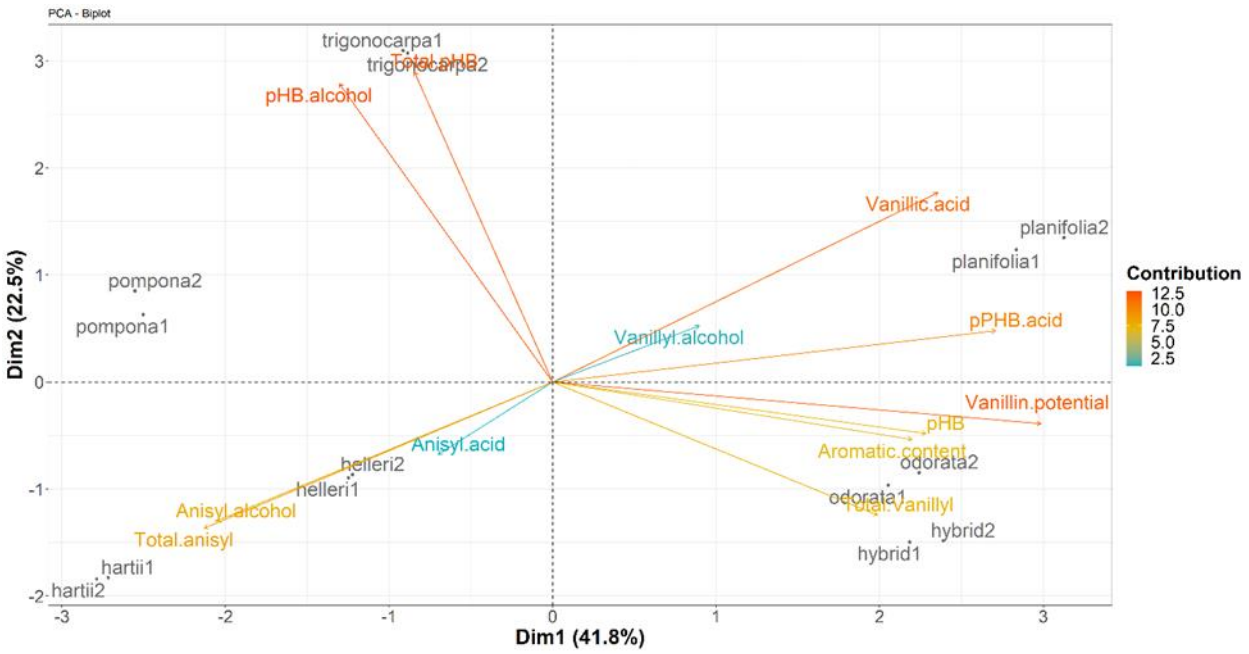

Fig. 5.8. *PCA plot displaying the aromatic compound composition among the studied Vanilla species, based on the eight volatile compounds (vanillin, vanillic acid, vanillyl alcohol, pHB, pHB acid, pHB alcohol, anisyl acid, anisyl alcohol) determined by HPLC.*

Table 5.6. Mean contents of main phenolic and anisyl compounds analysed by HPLC expressed in g·100 g^{-1} dry weight in mature vanilla pods.

***Vanilla* spp.**	**Vanillin potential[1]**	**Vanillyl family**			
		Glucovanillin	Vanillin	Vanillic acid	Vanillyl alcohol
V. hartii	0.000	0.000	0.000	0.000	0.338 ± 0.011
V. helleri	0.861 ± 0.001	0.085 ± 0.001	0.820 ± 0.018	0.000	0.089 ± 0.003
Hybrid	4.654 ± 0.091	0.901 ± 0.003	4.218 ± 0.093	0.077 ± 0.002	0.143 ± 0.002
V. odorata	5.553 ± 0.078	7.861 ± 0.165	1.748 ± 0.036	0.088 ± 0.008	1.333 ± 0.081
V. planifolia	6.731 ± 0.046	13.748 ± 0.069	0.076 ± 0.002	0.182 ± 0.010	0.232 ± 0.006
V. pompona	0.000	0.000	0.000	0.000	0.000
V. trigonocarpa	0.022 ± 0.003	0.000	0.022 ± 0.003	0.128 ± 0.003	0.877 ± 0.006

(continued)

***Vanilla* spp.**	**p-hydroxybenzyl family**			**Anisyl family**		**Total aromatic content**
	pHB[2]	pHB acid[3]	pHB alcohol[4]	Anisyl alcohol	Anisyl acid	
V. hartii	0.000	0.000	0.757 ± 0.036	4.757 ± 0.195	0.000	5.851 ± 0.241
V. helleri	0.035 ± 0.004	0.025 ± 0.002	0.645 ± 0.023	0.193 ± 0.007	0.340 ± 0.016	3.155 ± 0.103
Hybrid	0.298 ± 0.024	0.032 ± 0.001	0.240 ± 0.008	0.112 ± 0.003	0.000	5.556 ± 0.131
V. odorata	0.015 ± 0.001	0.036 ± 0.004	0.328 ± 0.014	0.127 ± 0.003	0.000	7.480 ± 0.027
V. planifolia	0.219 ± 0.022	0.078 ± 0.006	1.007 ± 0.004	0.000	0.000	8.449 ± 0.005
V. pompona	0.000	0.000	1.570 ± 0.092	1.584 ± 0.011	0.000	2.189 ± 0.055
V. trigonocarpa	0.000	0.015 ± 0.005	2.167 ± 0.011	0.000	0.000	3.209 ± 0.002

[1]0.484 times the content of glucovanillin added to the free vanillin content. [2]pHB = 4-hydroxybenzaldehyde; [3]pHB acid = 4-hydroxybenzoic acid; [4]pHB alchol = 4-hydroxybenzyl alcohol. Note: the high vanillin and low glucovanillin content in the pods of the hybrid was due to the high level of pod maturity when harvested.

5.5 DISCUSSION

The results obtained from this study indicate that the survival, vitality and early growth performance of vanilla plants depend on *Vanilla* species, land use system, light availability, and soil microbiota, as well as certain soil chemical properties and functional traits of onsite tree species. Furthermore, the aromatic compound composition of the vanilla beans varied among *Vanilla* species, and the obtained explorative aromatic profiles may serve as a stepping stone for further assessment of *Vanilla* CWR's market potential.

5.5.1 *Vanilla* plant performance: Survival, vitality, growth and earliness

The overall health performance of the hybrid and CWRs was higher compared to the crop species *V. planifolia*. As aforesaid, domestication and dispersion bottlenecks have resulted in a reduced intraspecific genetic variability (Bory et al., 2008), making *V. planifolia* highly susceptible to various abiotic and biotic stresses (Bhai & Thomas, 2000; Pinaria et al., 2010; Adame-Garcia et al., 2015). On the contrary, CWRs continue to evolve in the wild, developing traits to survive in changing environments (Dempewolf et al., 2017). *Vanilla pompona* and its hybrid with *V. planifolia*, for example, are known to be disease- and drought-resistant (Theis & Jimenez, 1957; Grisoni & Nany, 2021), with the latter being linked to the formation of lignified hypodermal cell walls and the production of phenolic root exudates (Koyyappurath et al., 2015), possibly explaining the outperformance of the vigorous *V. pompona* and hybrid in our study. A comprehensive study that examines the specific resistance potential of these and other *Vanilla* CWRs against climate and biotic stresses is required, using controlled experiments that involve imitations of future climate scenarios or pathogen inoculation, followed by the identification of associated resistance genes (R genes). Koyyappurath et al. (2015) suggested that the resistance mechanisms of vanilla plants are governed by distinct gene families, resulting in a multigenic resistance pattern. Further genome sequencing and R gene identification will be key to provide more insights herein (Chambers et al., 2020). On the other hand, the hybrid and *V. planifolia* outperformed the CWRs in terms of growth. Past selection of fast-growing *V. planifolia* varieties may explain this trend. The growth of *V. trigonocarpa* was generally lower compared to the other CWRs, possibly due to interspecific differences in plant functional traits, which in turn may be related to habitat-specific microenvironments (Restrepo et al., 2018). Studies comparing species' traits could give additional insights in the observed interspecific performance variability.

The health and growth performance of the individual *Vanilla* species depended on light (i.e. relative PAR, see 2.3.2) and land use system. *Vanilla hartii* performed better with limited light,

whereas the performance of the hybrid and *V. planifolia* increased with light intensity. Although less pronounced, the performance of *V. pompona* and *V. trigonocarpa* was also positively affected by light, while the latter did not seem to greatly affect *V. odorata*. The observed trend may be explained by the natural occurrence of *V. hartii* and *V. odorata* in the understory of old-growth forests, whereas *V. pompona* and *V. trigonocarpa* are found in open secondary forests and even wetlands (Householder et al., 2010; Soto Arenas & Dressler, 2010; Jimenez et al., 2017; Watteyn et al., 2020). However, we only examined the effects of onsite measured light intensity, which varied from 2 to 47 %. Díez et al. (2017) and Ko et al. (2020), for example, showed that *V. planifolia* performed best under intermediate radiation (above 17% and under 67%), with higher radiations affecting plant health and growth but stimulating production (Puthur, 2005). Additional experiments are thus required to support the observed species-specific light responses. The vitality of the hybrid and the CWRs did not differ across land use systems, whereas *V. planifolia* performed clearly inferior in SF compared to CP. This crop species has been domesticated for centuries (Bory et al., 2008), leading to a strong adaptation to agricultural areas, while, as mentioned before, CWRs may be more resilient to a wider range of habitats. The growth of both the hybrid and *V. planifolia*, however, was strongly reduced in SF compared to CP, which may be due to a combination of both reduced light conditions and other, non-measured variability among the two land use systems.

Soil bacterial diversity positively affected plant performance. Microorganisms play an important role in nutrient cycling, primary production, litter decomposition and climate regulation (Van Der Heijden et al., 2008; Delgado-Baquerizo et al., 2016), and microbial diversity has been associated with plant health and growth (Garbeva et al., 2004, 2006; Chaparro et al., 2012; Harman et al., 2019; Bertola et al., 2021). More specifically, vanilla disease-suppressive soils seem to be enriched with Actinobacteria, Armatimonadetes, Bacteroidetes, Firmicutes and Verrucomicrobiota (Xiong et al., 2015, 2017), explaining our observed positive effect of Firmicutes and Verrucomicrobiota on vanilla plant health. On the other hand, Firmicutes, Bacteroidetes and Acidobacteriota positively affected vanilla plant growth. Bacteroidetes play an important role in soil mineralisation (Fierer et al., 2007), and Firmicutes and Acidobacteria have been recognized as plant growth-promoting bacteria (Kielak et al., 2016; ; Kalam et al., 2020; Yoneda et al., 2021). Within the timeframe of this study, we were not able to analyse fungal diversity and composition, but Xiong et al. (2015, 2017) and Sandheep et al. (2014), for example, suggested that *Mortierella* and *Trichoderma* are vanilla pathogen suppressors, and a wide range of mycorrhizal fungi (e.g. *Ceratobasidium*, *Thanatephorus*, *Tulasnella*) have been associated with vanilla plant growth (Porras-Alfaro & Bayman, 2007; Flanagan & Mosquera-Espinoza, 2016; Johnson, 2021).

Moreover, plant performance was positively affected by soil organic matter and P, and negatively by low pH, K and Ca levels, which is conform with observations from La et al. (1998), Osorio et al. (2014) and Xiong et al. (2017). There likely exist feedback loops between soil properties and microbial communities, which in turn affect vanilla plant health and growth. As such, monitoring soil characteristics as part of the cultivation management scheme is crucial to assure optimal production conditions. Vanilla plant growth was affected by the SLA and leaf C:N ratio of tutor trees. Functional tree traits influence plant performance as they exert different effects on soil microbial communities and nutrient cycling (Gillespie et al., 2021). Tree species with high SLA, for example, increase soil fertility, while a high leaf C:N ratio immobilizes mineral nitrogen, thereby restricting its uptake (Hodgson et al., 2011; Brust, 2019). Selecting tutor trees based on specific functional traits may be an important aspect to take into account in vanilla cultivation designs, as has been previously recognized for cacao plantations (Sauvadet et al., 2019).

The vitality of the planted cuttings affected the condition of the newly developed shoots, indicating the importance to acquire healthy vegetative material when starting a vanilla plantation, as previously observed by Azofeifa-Bolaños et al. (2019). Especially the *V. planifolia* cuttings had a very low vitality compared to the other species. Although Grisoni & Nany, (2021) recommended a *V. planifolia* variety, designated as “Handa”, as being highly resistant, aromatic and with superior agronomic traits, and Azofeifa-Bolaños et al. (2014) established germplasm collections for the improvement of vanilla plant breeding in Costa Rica, the *V. planifolia* plants used in our study were derived from a local Costa Rican plantation and did not seem to resemble the above-defined features. Hence, it demonstrates the need to improve collaboration and exchange of healthy, certified vegetative material. Grisoni & Nany (2021), for example, proposed the addition of these varieties into the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), thereby facilitating access through the standard material transfer agreement (SMTA). Since our observations were based on visual assessments, the detected pathogen presence should be confirmed through molecular studies, and we highly recommend to examine pathogen presence prior to the exchange of vegetative material.

The CWRs *V. hartii*, *V. odorata*, and *V. pompona* started to flower less than two years after planting, coinciding with the flowering period of their wild populations (Soto Arenas & Dressler, 2010; personal observations). In commercial *V. planifolia* plantations, plants generally start to flower three to four years after planting (Havkin-Frenkel & Belanger, 2018). Earliness, i.e. the display of early flowering phenotypes, has been recognized as a highly desirable trait for breeding programs, if heritable (Li et al., 2020), as it may help farmers to expedite production and thus

income generation. The early flowering phenotypes should, however, be further linked with physiological and ontogenetic plant features to better understand the signals that trigger flowering.

5.5.2 Aromatic potential for market integration

We detected a range of aromatic compounds among the studied *Vanilla* species, whereby the hybrid, *V. odorata* and *V. planifolia* had the greatest vanillin potential. The high vanillin content of *V. planifolia* conforms its historical path as the widely used crop species. The vanillyl and pHB compound contents in the *V. planifolia* pods used in our study, however, seem to be higher compared to previous studies of Pérez Silva et al. (2021), confirming the formerly recognized intraspecific variability in aromatic potential of this crop species. Although *V. odorata* is the paternal parent of *V.* x *tahitensis* (*V. planifolia* x *V. odorata*) – a hybrid mainly cultivated in Indonesia and with unique aromatic features (Brunschwig et al., 2017) – this is the first study that quantified the aromatic potential of *V. odorata* itself. Vanillyl, pHB and anisyl compounds were found in both the hybrid used in this study ((*V. planifolia* x *V. pompona*) x *V. planifolia*) and *V. odorata*, indicating their high potential for market diversification. Moreover, our findings regarding the hybrid – widely cultivated in Costa Rica but not found on the international market (Havkin-Belanger et al., 2018) – correspond with former observations of Pérez-Silva et al. (2021). Although no vanillin was detected in *V. hartii* and *V. pompona*, anisyl alcohol was the major compound in both species, indicating its potential for specialty markets, as previously recognized by Maruenda et al. (2013) and Pérez-Silva et al. (2021) for *V. pompona*. They reported a variety of aromatic compounds for *V. pompona*, depending on accessions. For example, *V. pompona* growing in South America contains vanillin and has high anisyl alcohol contents, whereas no vanillin was detected in the Central-American *V. pompona* used in our study. The intraspecific variation, as also aforementioned for *V. planifolia*, may be due to regional differences in environmental conditions (Herrera-Cabrera et al., 2012; Pérez-Silva et al., 2021). *Vanilla helleri* contained a variety of compounds, yet, present in rather small quantities, and the same held for *V. trigonocarpa.*

Further research should quantify the aromatic compounds present in cured pods (i.e. post-harvest processing of mature green pods) to fully capture the species' aromatic potential. Moreover, the HPLC chromatograms of the CWRs showed peaks that may correspond to the presence of (non-aromatic) fatty acids or additional, non-identified aromatic compounds. Gas chromatography-mass spectrum analysis (GC-MS) compound identification is required to further expand our knowledge on the potential use of *Vanilla* CWRs in a variety of market niches. Although our

explorative study provides initial insights on the diversity of aromatic compounds present among the *Vanilla* CWRs and their potential to be used in different markets, integration of compelling CWRs will only be possible by transforming international market regulations. The latter are currently solely based on the quantification of vanillin potential to qualify vanilla beans.

5.6 CONCLUSIONS AND NEXT STEPS

Within the context of the SPASHA approach, we see great potential for integration of *Vanilla* CWRs, thereby diversifying the vanilla bean market. Based on above-discussed plant performance patterns, fallow or degraded lands could be reforested, followed by integration of *Vanilla* CWRs and the hybrid. Since we noticed practical difficulties using randomly available species within SF sites, tutor trees should ideally have a branching pattern that allows easy vanilla vine management and grow in the understory layer to facilitate pruning and thus light management. Furthermore, the effects of functional tree traits on plant performance should be taken into account during tutor tree selection. *Vanilla hartii* and *V. odorata* could be planted in the shadier zones, e.g. neighbouring the protected forests or under canopy tree species with denser crowns, while *V. pompona*, *V. trigonocarpa*, and the hybrid could be planted in zones with slightly higher light intensity. Existing agricultural lands could be diversified with whatsoever *Vanilla* species, following the same species-specific light gradient. Yet, a spatial transformation of current agricultural lands will be necessary. The traditional 3 m x 3 m cacao design, for example, will limit optimal crop growth and entangle proper management if to integrate vanilla and its tutor trees, as we already noticed the spatial competition among cacao and vanilla tutor trees. The implementation of sustainable production systems involving agroforestry, as proposed by SPASHA, coincides with the observed effects of soil microbiota and tree functional traits on vanilla plant performance; hence emphasizing the value of production systems that mimic the environmental conditions of vanilla's natural habitat, something that is far more challenging to establish in monocultures or artificial greenhouses.

A mixed *Vanilla* species design raises certain issues that require further evaluation of practical implementation. Interspecific variation in flowering seasons, for example, may increase organizational work load as farmers will need to pollinate and harvest several times a year. Species-specific habitat requirements may also complicate the planting and corresponding management scheme with regards to for example pruning or tutor tree selection. Abovementioned grouping of species demanding similar habitat conditions may facilitate a structured management. Furthermore, taking into account the current limitations on commercial *Vanilla* CWR use, only the

pods of the hybrid and *V. planifolia* can be harvested for commercial purposes. Continued research is highly required to complement our initial observations so as to properly integrate compelling *Vanilla* CWRs within a production context. First, controlled greenhouse experiments will enable the further selection of resistant CWRs, by examining the resistance against a broad range of abiotic and biotic stresses. Herewith, the identification of specific resistance genes will allow a more rapid recognition of robust varieties using molecular markers (previous studies of e.g. Herrera-Cabrera et al., 2012; Ramos-Castella et al., 2017; Hu et al., 2019; Hasing et al., 2020; Li et al., 2020; Chamber et al., 2021). Next, hybridization between compelling species and *V. planifolia* could greatly improve crop breeding programs, with the backcross (*V. planifolia* x *V. pompona*) x *V. planifolia* used in this study being a prominent example of a resistant hybrid with an attractive aromatic profile. We see great value in further hybridization of resistant (e.g. *V. pompona*) and aromatic (e.g. *V. odorata*, *V. hartii*) CWRs, all of which showed early flowering phenotypes, a trait of high agronomic value. Moreover, further research on phenology and fruit development as well as production quantification is required to provide recommendations within a production context, as we were only able to observe the first flowering period of early flowering phenotypes. Finally, facilitation of market integration will be key to enable the commercial use of new varieties, alongside a global transformation of market regulations and the commitment for product diversification.













6

Chapter 6

Exploring farmer preferences towards innovations in the vanilla supply chain

Adapted from:

Watteyn, C., Dejonghe, O., Van Hoyweghen, K., Azofeifa Bolaños, J.B., Karremans, A.P., Vranken, L., Reubens, B., Muys, B., Maertens, M. 2022. Exploring farmer preferences towards innovations in the vanilla supply chain. Journal of Cleaner Production. 330, Art.No. 129831. doi: 10.1016/j.jclepro.2021.129831

Photo: Surveys and workshops (agroforestry, vanilla pollination and cultivation) with farmers living in our study region in southwest Costa Rica. © Charlotte Watteyn

6.1 SUMMARY

The vanilla supply chain is characterized by high risk crop management and unstable supply. In order to meet global vanilla demand while improving production sustainability, new approaches considering agro-ecological cultivation systems and transparent supply chain management are needed. In this study, we aim to understand farmers' preferences towards such alternative vanilla cultivation and marketing systems, and thereby inform the development of a more sustainable vanilla supply chain. We implemented a discrete choice experiment and a survey with 186 farmers in the Península de Osa, southwest Costa Rica, and estimated mixed logit and latent class models. We find that surveyed farmers are in general positive about vanilla cultivation and prefer an agro-forestry system over vanilla cultivation in forests, crop wild relatives over commercial species, and manual pollination over natural pollination. Farmers prefer to sell green rather than cured vanilla beans, and like to engage in cooperatives that provide training and production contracts with buyers. Preferences are found to differ across farmers and we identify four preference classes. The preference class with the largest average probability (44%) shows the strongest preferences for agro-ecological vanilla cultivation in diversified agroforestry systems, using crop wild relatives and natural pollinators. Furthermore, this class has the highest likelihood to live in areas with restricted land use policies, creating opportunities for the development of more sustainable vanilla cultivation systems conform with the national program of landscape connectivity and biodiversity conservation. We propose a two-tier approach in alignment with observed farmer preferences and with potential for upscaling to similar areas along the Neotropics.

6.2 INTRODUCTION

Biodiversity, at genetic, species and ecosystem level, is a key element to achieve global food security, as it contributes to the long term stability of agricultural production and to the overall resilience of agroecosystems (Cramer et al., 2017; Pilling et al., 2020). Moreover, agricultural lands managed in a sustainable way contribute to a higher delivery of regulating ecosystem functions such as maintenance of water quality, soil moisture retention, carbon sequestration, and pollination (Schulte et al., 2014; Liebig et al., 2017). However, the increasing homogeneity of crop species and varieties, agricultural specialisation and intensification, and the conversion of natural habitats into monoculture landscapes are propelling habitat destruction and biodiversity loss, including loss of crop wild relatives (CWRs) (Khoury et al., 2014; Emmerson et al., 2016; Goettsch et al., 2021). CWRs are essential sources of a crop's genetic diversity (Meilleur and Hodgkin, 2004; Castañeda-Álvarez et al., 2016) and form the basis for future breeding programmes towards climate change adaptation, pest resistance and taste refinement (Maxted et al., 2013; Nair, 2019a, 2019b). Setting conservation priorities for CWRs and their natural habitats is therefore vital, along with the exploration of their potential agricultural use (Engels and Thormann, 2020).

Several crops are facing the above-mentioned problems related to genetic erosion and CWR loss, of which the high-value tropical spice vanilla is a noteworthy example. Vanilla is mainly extracted from the cured fruits, known as pods or beans, of *Vanilla planifolia* Jacks. ex Andrews, a perennial vine belonging to the section *Xanata* within the orchid genus *Vanilla* Plum. ex Mill. (Havkin-Frenkel and Belanger, 2018; Karremans et al., 2020). *Xanata* includes all fragrant *Vanilla* species, i.e. species producing fragrant beans, of which most are defined as endangered or critically endangered by the IUCN Red List (Goettsch et al., 2021). The earliest documented cultivation of vanilla beans dates back to the Totonac empire (Mexico), where naturally pollinated beans were traded for other products. After the Spanish conquest, however, some vegetative *Vanilla planifolia* cuttings were taken from its native regions in Mesoamerica to several European colonies in the Indian Ocean (Lubinsky et al., 2008, 2010). It was here that vanilla cultivation resulted in a real boom thanks to the discovery of a manual pollination technique in 1841 (Havkin-Frenkel and Belanger, 2018). The latter enables commercial production in absence of natural pollinators, but generally leads to over-pollination and decreases outcrossing rates (Flanagan and Mosquera-Espinosa, 2016). Moreover, since the leading production countries Madagascar and Indonesia (FAOSTAT, 2021) are not situated within the native region of fragrant *Vanilla* species (Neotropics), they depend on the limited genetic material from clonal descendants of these once

introduced vanilla cuttings. This, and the fact that vanilla is propagated in a vegetative way, has caused a genetic bottleneck that leads to increased susceptibility towards pathogen infestation and lower bean quality (Bory et al., 2008; Flanagan and Mosquera-Espinosa, 2016).

Besides cultivation problems, also the vanilla supply chain faces challenges. In Madagascar for example, a complex history of unsustainable practices, price volatility, low farm-gate prices, and collusion among traders characterizes the vanilla supply chain (Munshi, 2020). While international prices for cured vanilla beans reached record highs (> USD 600 / kg) during the past couple of years, Malagasy farmers continue to sell their cured vanilla beans at low prices (USD 8 to 34 / kg) and are living far below the international poverty line. Child labour and early harvest due to theft problems are common, leading to lower quality vanilla beans (MacIntosh, 2020). Furthermore, importing countries, such as the USA, Japan and European countries (ITC, 2020), re-export to other countries, but information about revenues and price margins along the vanilla supply chain is absent. The lack of quality control and transparency causes a situation of unstable vanilla supply, which is harmful to both producers and buyers.

New approaches and policies are needed to improve the overall resilience within the vanilla sector. In this context, more resilient cultivation methods and the organizational empowerment of small-scale vanilla farmers should be given major attention. For example, various fragrant *Vanilla* species can be found in their native region, possessing traits that are useful for crop improvement programs. These species can be seen as *Vanilla* CWRs since they have the potential to be used for aromatic profile diversification, pest resistance or climate change adaptation (Ranadive, 2010; Maruenda et al., 2013; Azofeifa-Bolaños et al., 2014; Flanagan and Mosquera-Espinosa, 2016; Havkin-Frenkel and Belanger, 2018). Moreover, natural cross-pollination by members of the bee tribe Euglossini has been reported for some of these species (Ackerman, 1983; Lubinsky et al., 2006; Householder et al., 2010; Soto Arenas and Dressler, 2014; Pansarin and Pansarin, 2014; Anjos et al., 2017; Watteyn et al., 2021), offering exciting opportunities to develop more sustainable agro-ecological systems that incorporate *Vanilla* CWRs and natural pollination. Overall, there is a need for effective *in situ* and *ex situ* conservation strategies of wild vanilla populations, protecting their natural habitat and associated ecological interactions, combined with community-based cultivation programs and supply chain management initiatives. This has been proposed by Flanagan and Mosquera-Espinosa (2016).

This study explores an innovative approach, combining joint land sparing/sharing (SPASHA) with supply chain coordination, to create a more sustainable and resilient vanilla system. Firstly, SPASHA combines two strategies in a single territory: (1) the protection of natural habitats

comprising wild vanilla populations, and (2) the transformation of neighbouring, degraded lands into agroforestry systems that incorporate the high-value cash crop vanilla (including its promising CWRs) (Watteyn et al., 2020). By mimicking the complexity of a natural ecosystem, these agroforestry systems serve as biological corridors for, among other, vanilla pollinators, while providing numerous ecosystem services as well as stable income generation via the diversity of included crops (Ghaley et al., 2014; Singh Tomar et al., 2021). Secondly, supply chain coordination, such as horizontal coordination among farmers in agricultural cooperatives and vertical coordination in contracts between farmers and buyers, can foster transparency and efficiency in food supply chains (Vroegindewey and Hodbod, 2018; Ba et al., 2019). The inclusion of small-scale farmers into supply chains of high value products such as vanilla, can have important effects towards rural income mobility and poverty alleviation (Wang et al., 2021).

To develop such initiatives, however, it is crucial to understand farmers' preferences ex ante - as has been documented for various farming and supply chain innovations (e.g. Blazy et al., 2011; Van den Broeck et al., 2017; Zhang et al., 2018; Feng and He, 2020). This study explores farmers' preferences towards potential innovations in vanilla production and marketing. We focus on the Península de Osa, a biodiversity hotspot of high conservation importance in southern Costa Rica where several *Vanilla* CWRs can be found (Karremans et al., 2020; Watteyn et al., 2020). Despite a successful track of forest conservation (Algeet-Abarquero et al., 2015), many of its protected areas remain isolated patches surrounded by fragmented landscapes (Sánchez-Azofeifa et al., 2003). One of the challenges in rural and impoverished areas such as the Península de Osa is therefore to improve habitat connectivity while realizing economic growth. The proposed approach to vanilla production and marketing could be one of the strategies that strives for a balance between biodiversity conservation and sustainable development.

Using a Discrete Choice Experiment (DCE), we aim to (1) study farmers' preferences for alternative vanilla cultivation and marketing systems, and (2) link the observed preferences to surveyed socio-economic and agronomic characteristics, as well as to farmer location within the surveyed area of the Península de Osa. Based on our findings, we propose a design for the first vanilla cultivation program in this region, with potential for upscaling to similar Neotropical areas. The development and subsequent implementation of such cultivation programs can contribute to the initial steps of creating a more inclusive and sustainable vanilla sector, characterized by applying environmental-friendly practices to assure production stability while supporting biodiversity conservation in and outside agricultural landscapes.

6.3 MATERIALD AND METHODS

6.3.1 Study area

The study is carried out in southwest Costa Rica. We focus on the Península de Osa, a peninsula that belongs to the Puntarenas province, and includes the districts Puerto Jimenez, Bahia Drake and Sierpe. It is one of the most biodiverse places on earth relative to its size, housing 2.5 % of the world's marine and terrestrial species (Kohlmann et al., 2010), and consists of the national park Corcovado, the forest reserve Golfo Dulce, and biological corridor Osa. The latter serves as a buffer zone connecting the protected areas (SINAC, 2018). The average annual precipitation ranges from 2,500 to 6,000 mm, and temperatures are on average 25 °C (Holdridge, 1967; Kappelle et al., 2003). The current main economic activities are ecotourism, agriculture, fishing, timber production and livestock farming (Hunt et al., 2015; Gutierrez et al., 2019).

6.3.2 Design of the choice experiment

Discrete choice experiments (DCEs), founded in random utility theory, are used in the field of agricultural economics to understand farmers' behaviour and preferences (Schipmann and Qaim, 2011; Abebe et al., 2013; Van den Broeck et al., 2017). They are consistent with the Lancaster economic theory of value (Lancaster, 1966), which assumes that respondents derive utility from a combination of properties (attributes) associated with a proposed scenario (alternative). It is assumed that respondents will choose the alternative that results in the highest utility (De Bekker-Grob et al., 2012). DCEs are the ideal technique for ex ante preference assessments, when data on revealed preferences are not (yet) available. We use a DCE to test farmers' preferences towards different types of vanilla cultivation and marketing systems, that represent more sustainable alternatives to the current situation. A series of hypothetical scenarios, differentiated by the levels of the included attributes, were presented to the farmers. Respondents were asked to choose their preferred scenario, allowing us to analyse farmers' preferences for specific attributes and the trade-offs farmers make between different attributes (Hanley et al., 2001).

Based on literature review, consultations with DCE experts and focus group discussions, we selected six attributes with different attribute levels: (1) vanilla cultivation system, (2) *Vanilla* species, (3) pollination technique, (4) supply chain coordination, (5) post-harvest processing of the green vanilla beans, and (6) annual revenues (Table 6.1). This represents a balanced attribute selection that allows us to focus on the essential elements of this study and does not overwhelm the respondents (Mangham et al., 2009). The first attribute, cultivation system, describes the type

of system used to cultivate vanilla. We distinguish two levels: (a) a secondary forest, in which vanilla is cultivated using the available trees as their tutor, or (b) a diversified agroforestry system in which vanilla is intercropped with other crops, and where nitrogen fixing tree species (*Erythrina* spp.) are used as vanilla tutor. The second attribute, vanilla species, refers to the use of either (a) *Vanilla* CWRs native to Costa Rica, or (b) the widely known crop species *V. planifolia*, and its hybrids with *V. odorata* C.Presl and *V. pompona* Schiede. The third attribute, pollination technique, describes the technique used to pollinate the vanilla flowers, which can be (a) by its natural pollinators, or (b) manually, the latter currently applied in commercial vanilla production. The fourth attribute, supply chain coordination, has three levels, whereby farmers can (a) work independently without contract or engagement in cooperatives, implying they educate themselves on the vanilla crop and search for suitable markets for the vanilla beans, (b) form part of a cooperative providing workshops and trainings related to vanilla cultivation, or (c) form part of a cooperative providing the aforementioned trainings as well as holding a contract with a buyer for the marketing of vanilla. The fifth attribute, post-harvest process, refers to the final product sold by the farmer, which can be (a) the unprocessed green vanilla bean, sold right after harvest, or (b) the cured vanilla bean, being the result of a curing process (Havkin-Frenkel and Belanger, 2018). The last attribute, annual revenue, is a continuous and monetary attribute and expressed in *US Dollar* (USD), which is, besides the local currency *Costa Rican Colon* (CRC), a dominantly used currency. A sufficient number of revenue levels is needed to determine the relationship between the revenue and its derived utility. We chose five levels, ranging from USD 1,000 to 30,000 per hectare and based on different production scenarios and farm-gate prices.

We developed choice cards consisting of two unlabelled alternatives and a status-quo option (opt-out), the latter indicating that the respondent is not interested in the proposed alternatives (Hensher et al., 2012; Kuhfeld, 2011). As we chose for two alternatives, each having six attributes and associated levels, the number of possible choice cards was high (2^4x3x5). Hence, a fractional factorial design was applied, selecting only a subset of the total number of choice tasks. We chose the most efficient design using the D-efficiency criterion, aiming to minimise the standard error of the parameter estimates for a given sample size and avoiding the inclusion of less meaningful choice sets (Choicemetrics, 2018). Small positive and negative priors (0.001 and -0.001) were used to increase the design efficiency. For the *Vanilla* species, pollination technique and post-harvest process attributes, small negative priors were added. Positive priors were added for the cultivation system, supply chain coordination and revenue attributes. Four constraints were added to the design to ensure realistic alternatives (Table A5.1). Using Ngene, a matrix was composed of values that represent the attribute levels, resulting in three blocks of ten choice cards each.

Table 6.1. *Overview and description of selected attributes and corresponding levels.*

Attribute name	Attribute description	Attribute levels
Cultivation system	Cultivation system in which vanilla is produced	(a) Secondary forest (b) Intercropping agroforestry systems
***Vanilla* species**	*Vanilla* species that is used for production	(a) *Vanilla* CWRs (b) Commercial *Vanilla* species
Pollination technique	Type of pollination technique that is used in vanilla production	(a) Natural pollination (b) Manual pollination
Supply chain coordination	Engagement in cooperatives providing training on vanilla production and in contracting with buyers for a guaranteed market outlet	(a) Independent (no cooperatives nor contract) (b) Cooperative providing training (c) Cooperative providing training and market outlet
Post-harvest process	Level of post-harvest processing by farmer and type of product sold	(a) Green vanilla beans (b) Cured vanilla beans
Annual revenue[1]	Annual net revenue per hectare from vanilla production	$ 1,000, $ 4,500, $ 7,500, $ 15,000, $ 30,000

[1]calculated using the following data: estimated number of vanilla plants and vanilla beans per hectare in different scenarios of cultivation systems, *Vanilla* species, pollination techniques, supply chain coordination and post-harvest processing, taking into account average green/cured vanilla bean weight, production costs and vanilla bean prices.

6.3.3 Hypotheses

Based on the economic situation of our study area, we hypothesize a positive attitude of farmers towards the high-value cash crop vanilla. More specifically, we expect a preference for intercropped systems, commercial *Vanilla* species, manual pollination, farmers' cooperatives, and selling cured vanilla beans. These hypotheses are based on the assumption that farmers would trade-off a higher current return or profit from traditional production methods for the resilience and potential higher long-term return from agro-ecological practices. Yet, we expect preferences to be heterogeneous across farmers as they might make different trade-offs depending on their socio-economic situation.

6.3.4 Data collection

The choice experiment was conducted along with a complementary survey in the period August-September 2019, with a total of 186 respondents. The respondents were defined as the farmers living in the Península de Osa, and identified using databases of the farmer cooperative Osacoop R.L. and the Ministerio de Agricultura y Ganadería (MAG). We additionally used an exponential non-discriminative snowball sampling (Bhat, 2019) for farmers not associated to one of the aforementioned institutions. Due to logistical constraints, some villages could not be sampled. Yet, our sample size represents about 20 % of the total farmer population (± 1000) within the peninsula (INEC, 2014), providing a rather large sampling ratio. The sample size exceeds the minimum required sample size to carry out a reliable DCE based on our selected attributes and corresponding levels, using the methodology described in de Bekker-Grob et al. (2015).

The DCE was preceded by a farmer survey in order to collect information about farmer's characteristics (Table A5.2). This information is crucial to reveal whether and how respondents' choices depend on their characteristics (Hensher et al., 2012). SurveyCTO, a data collection platform, was used to register the answers of the respondents to both the DCE and the Farmer Survey. Before the interview, an introduction video with information on the vanilla crop, its management, pollination, post-harvest processing, and current cultivation and market situation was shown to the respondents to ensure they were all equally informed.

Illiteracy of respondents in rural areas can complicate the implementation of a DCE (Mangham et al., 2009; Bennett and Birol, 2010). Therefore, a graphical representation of all choice cards was created based on the matrix with two alternatives (and their respective attribute levels) and one opt-out option (Fig. 6.1). The respondents were shown ten choice cards and were asked to indicate their preferred alternative on each card. It took maximum 30 minutes to complete the

survey, limiting fatigue bias. In addition, we varied the order in which the choice cards of a block were presented to the respondents to avoid bias related to learning effects (Van den Broeck et al., 2017). Before implementation of the DCE, we carried out a pilot study to ensure that respondents and enumerators clearly understood the concept and the attributes and levels on the choice cards. To account for possible cultural and language differences between researchers and respondents, the latter were questioned in their mother tongue by two local enumerators, trained by the authors. The purpose of the study, the procedure of the DCE (without wrong or right answers) was clearly explained to the respondents, and their anonymity was guaranteed.

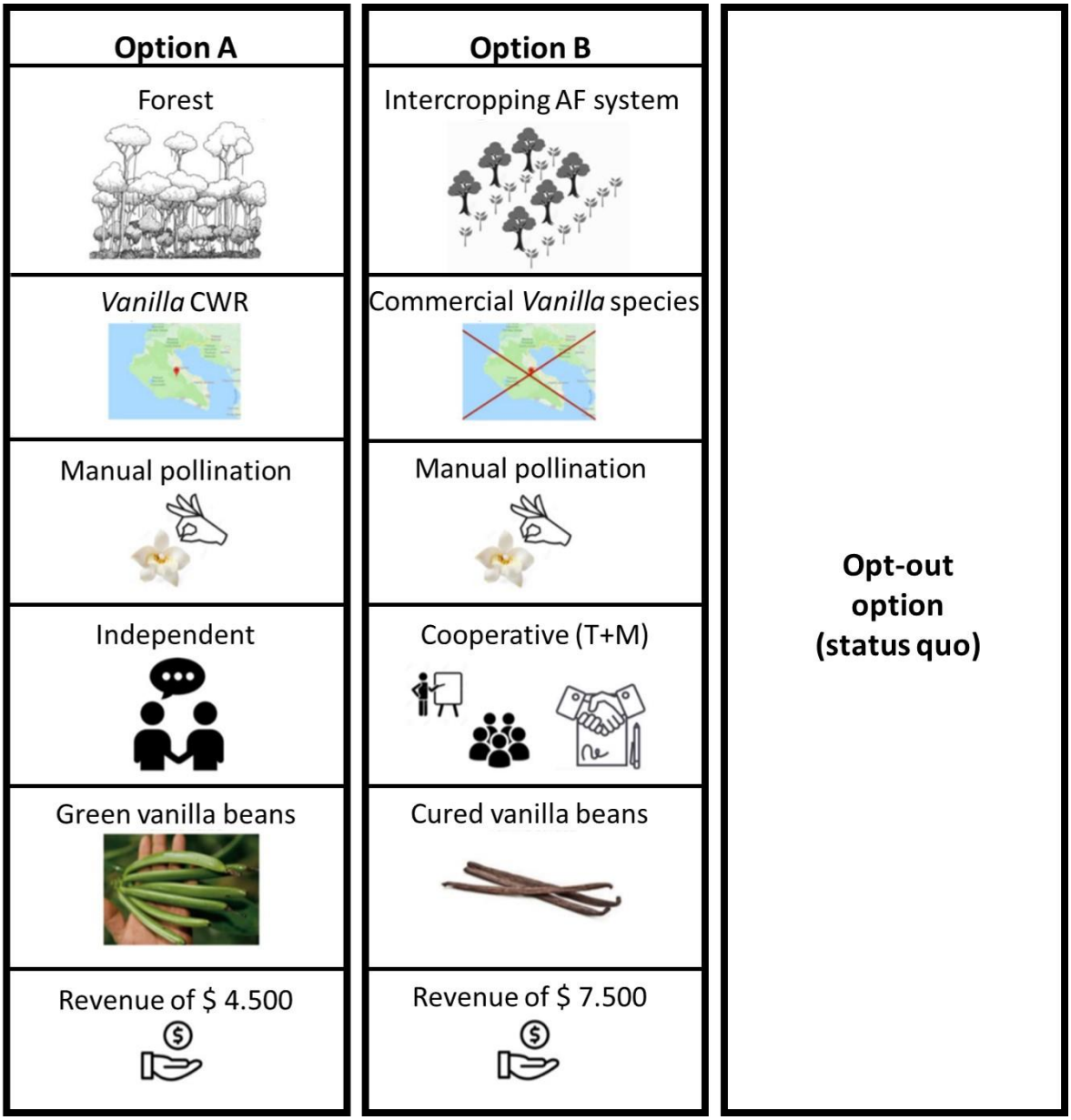

Fig. 6.1. *Example of a choice card used during the DCE, showing the two alternatives (option A and B), differentiated by the levels of the six attributes, and an opt-out option (status-quo).*

6.3.5 Econometric models

We estimated a mixed logit (MXL) model with random coefficient specification (McFadden and Train, 2000), involving the six attributes. In order to get the final parameter estimates and to provide a sufficient balance between parameter estimate stability and estimation time, 500 Halton draws were used. We further explored preference heterogeneity using latent class (LC) models. These models assume a specific number of latent classes in the population, with preference homogeneity within and preference heterogeneity across latent classes (Greene and Hensher, 2003). LC models identify systematic heterogeneity by simultaneously estimating the probability of a respondent to belong to a certain class, depending on respondents' characteristics and the probability that a certain alternative is chosen. The probability that an individual (i) chooses an alternative (j) from a set of alternatives (C) conditional on the individual belonging to a latent class (l) takes the conditional logit form: $P_{ij/l} = \frac{\exp(V_{ij/l}(Z_{j/l}, X_{i/l}))}{\sum_{h=1}^{C} \exp(V_{ih/l}(Z_{h/l}, X_{i/l}))}$.

The number of latent classes was determined by evaluating the statistical decision criteria (Akaike information criterion - AIC, and Bayesian information criterion - BIC) of the LC model. We selected a model with four latent classes. After estimating the LC model, we used the estimated LC probabilities and the maximum membership probability criterion (cpmax) to assign respondents to mutually exclusive preference classes, and further describe heterogeneity by comparing respondent's characteristics across these classes. In addition, maps visualizing the distribution of the preference classes within our study area were created using QGIS (3.16.0).

6.4 RESULTS

6.4.1 Descriptive statistics of the respondents

About 73% of the respondents are male (Table A5.2). The majority (61%) of the respondents are between 25 and 50 years old. About 85% did not receive any education or only went to primary school. Furthermore, we observe that 59% of the respondents have a gross monthly income lower than the minimum wage of 250,000 CRC, based on the minimum daily wage of a non-skilled worker (Ministerio de Trabajo y Seguridad Social, 2021). About 80% are producing crops, 50% are breeding animals, 12% are involved in reforestation programs and 7% are not using their land. Dominant crops are oil palm (45%), fruits (33%), vegetables (11%) and cacao (9%). About 43% are cattle farmers. About 32% of the respondents are subsistence-oriented, while the remaining are predominantly commercially-oriented; with 38% selling on the local market, 24% on the national, and 14% on the international market, and 9% does not know the destination. About 30%

of the respondents are member of a cooperative, whereas 70% are working independently. Finally, 63% of the respondents possess knowledge on the vanilla crop, with only one commercializing vanilla beans.

6.4.2 Mixed logit model

The results of the basic MXL model are reported in Table 6.2. The alternative specific constant (ASC) parameter is significantly positive (0.95, P < 0.01) , meaning that farmers experience an increase in utility when moving away from their status quo to a situation in which they would cultivate vanilla under the proposed scenario's for production and marketing.

Table 6.2. *Estimation results from the basic MXL model.*

Attribute name	Mean Parameter estimate (SE)	Standard deviation estimate (SE)
ASC	0.95***	3.09***
		(0.36)
Cultivation system		
Intercropping agroforestry systems	0.52***	0.89***
	(0.11)	(0.15)
***Vanilla* species**		
Commercial *Vanilla* species	-0.21***	0.30
	(0.07)	(0.26)
Pollination technique		
Manual pollination	0.27***	-0.72***
	(0.10)	(0.16)
Supply chain coordination		
Cooperative (training)	0.36***	0.29
	(0.11)	(0.25)
Cooperative (training + contract)	0.81***	-1.35***
	(0.16)	(0.16)
Post-harvest process		
Cured vanilla beans	-0.31***	-0.50***
	(0.08)	(0.10)
Annual revenue	0.03*	-0.00
	(0.02)	(0.03)
Log likelihood	-1579.49	
Chi2	117.26	
AIC	3190.98	
BIC	3297.01	

Significance shown at * p < 0.1, ** p < 0.05, *** p < 0.01. Number of observations = 5580.

Overall, farmers show a preference towards the cultivation of vanilla within intercropping agroforestry systems (0.52, P < 0.01), using *Vanilla* CWRs (-0.21, P < 0.01) and applying manual pollination techniques (0.27, P < 0.01). Furthermore, they prefer to be part of a cooperative (0.36, P < 0.01), with stronger preferences towards a cooperative providing training and a contract with buyers (0.81, P < 0.01), and to sell green vanilla beans rather than cured beans (-0.31, P < 0.01).

We observe a significantly positive coefficient for the revenue attribute (0.03, P < 0.1), demonstrating a preference towards higher revenues. The standard deviations of the ASC parameter as well as the production system, pollination technique, supply chain coordination, and post-harvest process are significant, implying heterogeneity among farmers in the preferences for these attributes.

6.4.3 Latent class model

The results of the LC model are reported in Table 6.3. The ASC estimates are significantly positive in latent classes 1 and 2, implying farmers prefer vanilla cultivation above their status quo. Within latent class 1, farmers are indifferent regarding the cultivation system or species used, but show strong preferences towards manual pollination, leading to an ensured vanilla bean yield. There is a preference to work independently and to sell green vanilla beans. In contrast, in latent class 2, there is a preference in favour of intercropping agroforestry systems, involving vanilla CWRs and natural pollinators, to be part of a cooperative and to sell green vanilla beans.

Table 6.3. *Estimation results from the LC model with four latent classes.*

	Mean parameter estimate (SE)			
Attribute name	**Class 1**	**Class 2**	**Class 3**	**Class 4**
ASC	1.30**	2.54***	-2.41***	-2.51***
	(0.57)	(0.70)	(0.85)	(0.45)
Cultivation system				
Intercropping agroforestry system	-0.12	0.63***	-0.69*	1.68***
	(0.17)	(0.12)	(0.38)	(0.33)
***Vanilla* species**				
Commercial *Vanilla* species	0.01	-0.23**	-0.53	-0.33
	(0.13)	(0.09)	(0.33)	(0.22)
Pollination technique				
Manual pollination	0.59***	-0.16*	-0.59*	0.95***
	(0.19)	(0.09)	(0.36)	(0.22)
Supply chain management				
Cooperative (training)	-0.39*	0.73***	1.21*	0.08
	(0.21)	(0.15)	(0.66)	(0.25)
Cooperative (training + contract)	-0.48**	1.31***	4.19***	0.04
	(0.24)	(0.20)	(0.73)	(0.25)
Post-harvest process				
Cured vanilla beans	-0.34**	-0.31***	0.44	-0.31
	(0.15)	(0.10)	(0.32)	(0.21)
Annual revenue	0.05	0.03	0.06	0.02
	(0.015)	(0.07)	(0.05)	(0.02)
Log likelihood	-1552.7			
AIC	3175.5			
BIC	3407.4			

Significance shown at * p < 0.1, ** p < 0.05, *** p < 0.01. Number of observations = 5580.

The ASC estimates are significantly negative in latent classes 3 and 4, implying farmers do not perceive vanilla cultivation as an improvement upon their status quo. Within latent class 3, there is a preference to cultivate vanilla within secondary forests with natural pollinators. This class also shows a strong preference towards cooperatives, especially if providing training and a guaranteed market, but no preferences towards the vanilla species nor the post-harvest process of the vanilla beans. Within latent class 4, we find significant coefficients for intercropping agroforestry systems and the application of manual pollination, pointing to preferences for these attributes, but not for vanilla species, supply chain management, or post-harvest process.

Using results from the LC model, we assign farmers to mutually exclusive preference classes based on the maximum membership probability and compare respondents' characteristics across the four classes using t-tests to reveal significant differences in characteristics between the four classes (Table 6.4). Class 1 (24.4% of respondents) involves the highest percentage of farmers who did not receive any form of education, and are the least active in agroforestry practices and palm oil production. These - generally younger - farmers are more focused on self-subsistence farming, with slightly higher percentages of fruit and vegetable production. If they sell their products, they predominantly do so on local markets in an independent way. Class 2 (44.8% of respondents) consists of farmers who mainly received primary school education. This class includes the highest percentages of cacao and oil palm as well as livestock farmers. These farmers mainly sell their products, principally on local but also (inter)national markets. Class 2 farmers are the ones most involved in cooperatives and principally located in the forest reserve or biological corridor.

Class 3 is the smallest class (9.6% of respondents) and includes the oldest and poorest farmers, with significantly more knowledge on the vanilla crop compared to the other classes. This class has the lowest percentages for each of the included farming activities. These farmers are mainly self-subsistence oriented, but the products they trade are largely sold at (inter)national markets. Lastly, class 4 (21.1% of respondents) consists of younger farmers with the highest education and income levels, and the largest farm areas. Their dominant activities are livestock farming and palm oil production. They work most often in an independent way, are commercially oriented, mainly sell on (inter)national markets, and are more likely to be located outside the forest reserve or biological corridor. Class 4 farmers are significantly less informed about the vanilla crop compared to the other three classes. Since only 27 % of the questioned farmers are female, all four classes show a high percentage of male respondents. Finally, farmers of class 1, 2 and 3 are equally involved in reforestation programs, whereas reforestation is not a priority for class 4.

The four preference classes are represented on the map of our study area to reveal the distribution of the different types of farmers within the local context (Fig. 6.2). From this, we observe that there is a mix of farmers form different preference classes spread across the area. However, farmers from class 2, and to a lesser extent farmers from class 3, are dominantly living in the forest reserve and/or biological corridor. Farmers from class 1 and 4 are located more outside important conservation areas.

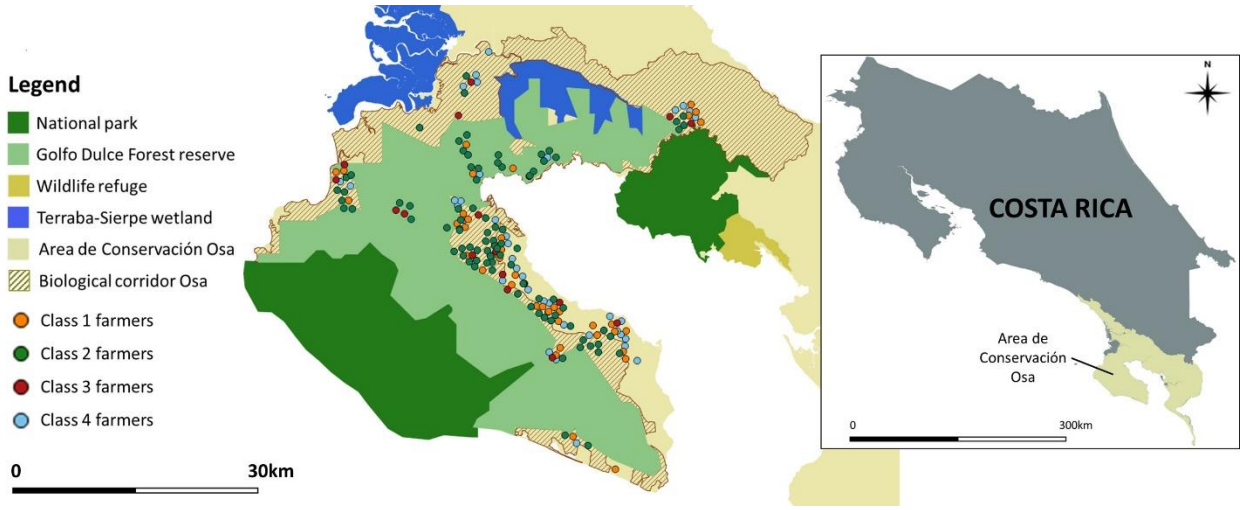

Fig. 6.2. *Map showing the distribution of the four farmer classes within the surveyed area.*

Table 6.4. *Comparison of farmer characteristics across four mutually exclusive preference classes.*

	Mean parameter estimate (SE)				Comparison between individual classes (t-tests)					
Class share	**Class 1 (24.4%)**	**Class 2 (44.8%)**	**Class 3 (9.6%)**	**Class 4 (21.1%)**	**1 & 2**	**1 & 3**	**1 & 4**	**2 & 3**	**2 & 4**	**3 & 4**
Socio-economic characteristics										
Gender (male)	0.71	0.76	0.78	0.65	-2.76***	-2.80***	3.43***	-1.09	6.80***	5.37***
	(0.45)	(0.43)	(0.42)	(0.48)						
Age (> 50 years)	0.60	0.65	0.78	0.60	-3.38***	-7.55***	-0.24	-6.73***	3.04***	7.32***
	(0.49	(0.48)	(0.42)	(0.49)						
Education										
No education	0.36	0.23	0.22	0.20	8.20***	5.69***	8.80***	0.52	2.24**	1.06
	(0.48)	(0.42)	(0.42)	(0.40)						
Primary school	0.48	0.69	0.56	0.55	-12.82***	-3.09***	-3.67***	5.87***	8.20***	0.22
	(0.50)	(0.46)	(0.50)	(0.50)						
Secondary school	0.17	0.06	0.22	0.15	11.00***	-2.80***	1.13	-12.64***	-9.42***	3.70***
	(0.37)	(0.23)	(0.42)	(0.36)						
University	0.00	0.02	0.00	0.10	-5.48***	-	-11.83***	3.58***	-10.46***	-7.74***
	(0.00)	(0.15)	(0.00)	(0.30)						
Income										
< 250.000 CRC	0.64	0.59	0.83	0.40	2.97***	-8.23***	12.42***	-10.74***	11.25***	18.30***
	(0.48)	(0.49)	(0.37)	(0.49)						
250.000-500.000 CRC	0.29	0.30	0.11	0.30	-1.06	8.17***	-0.78	9.23***	0.14	-8.70***
	(0.45)	(0.46)	(0.31)	(0.46)						
500.000-750.000 CRC	0.02	0.00	0.00	0.05	7.93***	3.63***	-3.47***	-	-11.65***	-5.33***
	(0.15)	(0.00)	(0.00)	(0.22)						
> 750.000 CRC	0.00	0.05	0.00	0.03	-7.84***	-	-5.68***	5.13***	3.16***	-3.72***
	(0.00)	(0.21)	(0.00)	(0.16)						
No information	0.05	0.06	0.06	0.23	-1.35	-0.71	-13.36***	0.23	-15.68***	-8.84***
	(0.21)	(0.23)	(0.23)	(0.42)						
Characteristics related to farming activities										
Household help	0.31	0.28	0.33	0.38	1.96*	-0.99	-3.43***	-2.53**	-5.96***	-1.67*
	(0.46)	(0.45)	(0.47)	(0.48)						
Cacao	0.05	0.15	0.00	0.05	-9.47***	5.19***	-0.27	9.80***	9.03***	-5.33***
	(0.21)	(0.36)	(0.00)	(0.22)						
Palm	0.31	0.57	0.39	0.38	-15.62***	-3.28***	-3.43***	7.74***	11.33***	0.55
	(0.46)	(0.50)	(0.49)	(0.48)						
Fruits + vegetables	0.38	0.34	0.17	0.35	2.67***	9.16***	1.59	7.88***	-0.77	-7.91***
	(0.49)	(0.47)	(0.37)	(0.48)						
Livestock	0.40	0.45	0.28	0.45	-2.86***	5.16***	-2.27**	7.58***	0.20	-6.68***

	(0.49)	(0.50)	(0.45)	(0.50)						
Reforestation	0.14	0.14	0.17	0.05	0.28	-1.30	7.85***	-1.63	8.22***	8.17***
	(0.35)	(0.35)	(0.37)	(0.22)						
Farm area	0.38	0.43	0.28	0.50	-2.91***	4.22***	-5.99***	6.61***	-4.02***	-8.85***
(> 25 ha)	(0.48)	(0.49)	(0.45)	(0.50)						
Agroforestry	0.57	0.64	0.61	0.63	-4.08***	-1.57	-2.71***	1.25	0.86	-0.55
	(0.49)	(0.48)	(0.49)	(0.48)						
Characteristics related to marketing										
Product purpose	0.59	0.71	0.56	0.75	-7.11***	1.57	-8.27***	7.03***	-2.60***	-8.26***
(selling)	(0.49)	(0.45)	(0.50)	(0.43)						
Product destination	0.31	0.34	0.50	0.48	-1.72*	-7.81***	-8.53***	-7.20***	-8.19***	0.97
((inter)national)	(0.46)	(0.47)	(0.50)	(0.50)						
Farmers' cooperative	0.26	0.37	0.28	0.20	-6.83***	-0.70	3.65***	4.17***	10.74***	3.61***
	(0.44)	(0.48)	(0.45)	(0.40)						
Farmer location										
Forest reserve + corridor	0.62	0.78	0.72	0.55	-10.59***	-4.22***	3.48***	2.85***	14.80***	6.88***
	(0.49)	(0.41)	(0.45)	(0.50)						
Forest reserve	0.19	0.31	0.22	0.10	-8.13***	-1.54	6.40***	4.25***	14.61***	6.93***
	(0.39)	(0.46)	(0.42)	(0.30)						
Vanilla knowledge										
Vanilla knowledge	0.38	0.38	0.44	0.30	-0.17	-2.52**	4.25***	-2.63***	5.02***	5.92***
	(0.49)	(0.49)	(0.50)	(0.46)						

Significance shown at * p < 0.1, ** p < 0.05, *** p < 0.01

6.5 DISCUSSION

6.5.1 Farmer preferences regarding vanilla cultivation

The results show that most farmers of the Península de Osa perceive vanilla cultivation as an improvement upon their current situation. The results of the MXL model demonstrate a high interest to cultivate vanilla in diversified agro-ecological systems, using CWRs and involving natural pollinators. Farmers are found to prefer selling green over cured vanilla beans, and to like engaging in vanilla cooperatives that provide training and production contracts with buyers. Yet, preferences are found to be heterogeneous across farmers, and with the LC model we identify four classes with distinct preferences. This heterogeneity is correlated with agricultural characteristics such as location, farming activities and marketing, as well as socio-economic characteristics such as education and income level.

Classes 1 and 2 include farmers who are willing to cultivate vanilla. Class 1 farmers prefer to apply manual pollination and to work independently, whereas class 2 farmers prefer to involve natural pollinators, to use *Vanilla* CWRs within agroforestry systems, and to form part of a cooperative. Since both classes include farmers characterized by rather low income levels, agricultural diversification can be a mechanism for poverty alleviation, as proposed by Feliciano (2019). On-farm diversification, defined as the integration of (cash) crops, livestock, and/or trees (Altieri et al., 2015; Hufnagel et al., 2020), can act as a safety net in the event of price fluctuations or other disruptions to value chains (van Zonneveld et al., 2020). Although the risks and barriers related to agricultural adaptation might be higher for relatively poor households with resource constraints (Brown et al., 2019), this farmer group seems to be willing to take the risk. An interesting observation is the participation of both classes in cacao, oil palm, and livestock farming, with class 2 being significantly more involved in all three activities. The preference of class 2 farmers towards CWRs might originate from past negative experiences regarding pests and diseases, such as frosty pod rot (*Moniliophthora roreri*) in cacao and bud rot in oil palm (Alvarado et al., 2013; Bailey et al., 2018). Asrat and Simane (2018) and Pant et al. (2012) observed that farmers greatly value crop traits related to yield stability and environmental adaptability. The breeding potential of different vanilla varieties thus definitely merits further investigation.

Classes 3 and 4 include farmers with negative attitudes towards vanilla cultivation. Class 3 involves the oldest, most experienced farmers with more knowledge about the vanilla crop. The Península de Osa has a brief but tragic history of vanilla cultivation, due to a regional fungi attack

and the lack of resistant varieties (Azofeifa-Bolaños et al., 2014). It is therefore possible that some of these farmers have been cultivating vanilla in the past (or at least have heard about it), which might explain their negative attitude. Class 4 includes farmers who focus on livestock and oil palm to be sold at (inter)national markets, and have larger farm areas. They are the most wealthy farmers with the highest education levels. As observed by Lestari et al. (2019) and Abdullah et al. (2021), large-scale cattle farmers don't seem to perceive biosecurity or innovation incentives as a real advantage. They can sell their cattle at any time, giving them a secure business –as confirmed through communication with Universidad Nacional de Costa Rica and local cattle farmers.

6.5.2 Location of potential vanilla farmers within the landscape

The observation of a strong positive attitude towards agro-ecological cultivation systems of almost half of the questioned farmers is remarkable. Their attitude is especially noteworthy considering the high conservation value of their location within the Península de Osa, i.e. mainly in areas bordering protected areas and serving as corridors between forest patches where native vanilla species and their natural pollinators are present. Since they are living in areas with a stricter land use policy, the development of sustainable practices is key and seems to be in alignment with observed preferences. In the case of vanilla, we see a clear added value of the proposed SPASHA approach, whereby current degraded lands, pastures or monocultures could be transformed to agroforestry systems functioning as biological corridors between forest patches. This approach is in line with farmers' preferences, improves production sustainability, and provides multiple ecosystem services and environmental benefits since they (1) transform degraded areas into agroforestry systems for vanilla cultivation, potentially using promising CWRs, and (2) protect surrounding forests necessary to preserve natural vanilla populations and to maintain vital ecological interactions such as pollination (Jose, 2009; van Zonneveld et al., 2020).

Furthermore, farmers who prefer more agro-ecological systems also show a strong preference towards a vanilla cooperative, especially one providing training and a market outlet. Previous studies (Kolade and Harpham, 2014; Ahmed and Mesfin, 2017; Manda et al., 2020; Olagunju et al., 2021) showed that cooperative membership improves technical assistance and benefits farmers in various ways, especially those farmers with less market access and a lack of market awareness. Benefits of cooperative membership, however, highly depend on cooperative type (Verhofstadt and Maertens, 2015). Yet, cooperatives might play a crucial role in farmers' adoption of sustainable vanilla cultivation methods, especially for farmers with a more secluded location

and interest in agro-ecological systems. On the contrary, farmers who are living closer to the villages and outside land use restriction areas mainly prefer to work independently. Access to potential processing units and buyers is probably more straightforward for these farmers, and the implementation of agricultural activities has less restrictions, explaining their preference towards direct contracts with vanilla buyers. They could build on their already established market network used for the trade of current farm products.

6.5.3 Proposition for innovation in vanilla cultivation

Our findings have important and direct implications for the development of a vanilla cultivation program within the Península de Osa. Considering the distinct differences in preferences among the surveyed farmers with interest towards vanilla cultivation, we propose a two-tier approach (Fig. 6.3). This approach consists of the joint or consecutive establishment of (1) a mainstream system, using current commercial vanilla varieties and applying manual pollination, involving independent farmers living outside protected areas, and (2) an agro-ecological or SPASHA system, using *Vanilla* CWRs and natural pollinators, involving farmers being part of a cooperative and living inside areas with strict land use policies.

At present, only the crop species *Vanilla planifolia* and its hybrids with *V. pompona* and *V. odorata* are allowed for commercialization (Havkin-Frenkel and Belanger, 2010, 2018; Hu et al., 2019), and the present knowledge gap regarding pollinator species and pollination effectiveness within a production context favours the application of manual pollination. Besides, no institutional support towards the vanilla crop is currently provided in the area. Due to these restrictions, direct implementation will be easier for farmers with preferences coinciding the mainstream approach. They would need to educate themselves on the management of the vanilla crop and directly connect to vanilla buyers. Our results, as discussed in Section 4.2, show that surveyed farmers possessing the above-mentioned preferences are living closer to the larger villages and just outside the forest reserve or corridor. Here, a more flexible land use policy is in place that facilitates the implementation of new activities. The largest part of the questioned farmers, however, prefers *Vanilla* CWRs, intercropping agroforestry systems, natural pollinators, and a cooperative providing training and a market outlet. Therefore, further research could focus on (1) the effectiveness or benefits of natural pollinators within a production context, (2) the potential, both production- and market-wise, of *Vanilla* CWRs and their legalization for sustainable use, (3) the development of agroforestry systems that allow successful integration of the vanilla crop and its pollinators, and (4) the creation of a vanilla cooperative organizing workshops and trainings to

support farmers' adoption. Once there is more information available about these CWRs, besides an approved legalization for commercialization, CWR germplasm could be used for crop improvement programs and exposed to the market. The same holds for the involvement of natural pollinators. Few studies tackling this topic observed low fruit sets in natural vanilla populations (Gigant et al., 2016; Watteyn et al., 2021), favouring manual pollination. However, naturally pollinated vanilla beans might attract specialty markets and thereby higher prices (personal communication with vanilla farmers of Veracruz, Mexico). This approach should be supported through national policies of CWR conservation and community-based cultivation programs, as proposed by Flanagan & Mosquera-Espinosa (2016).

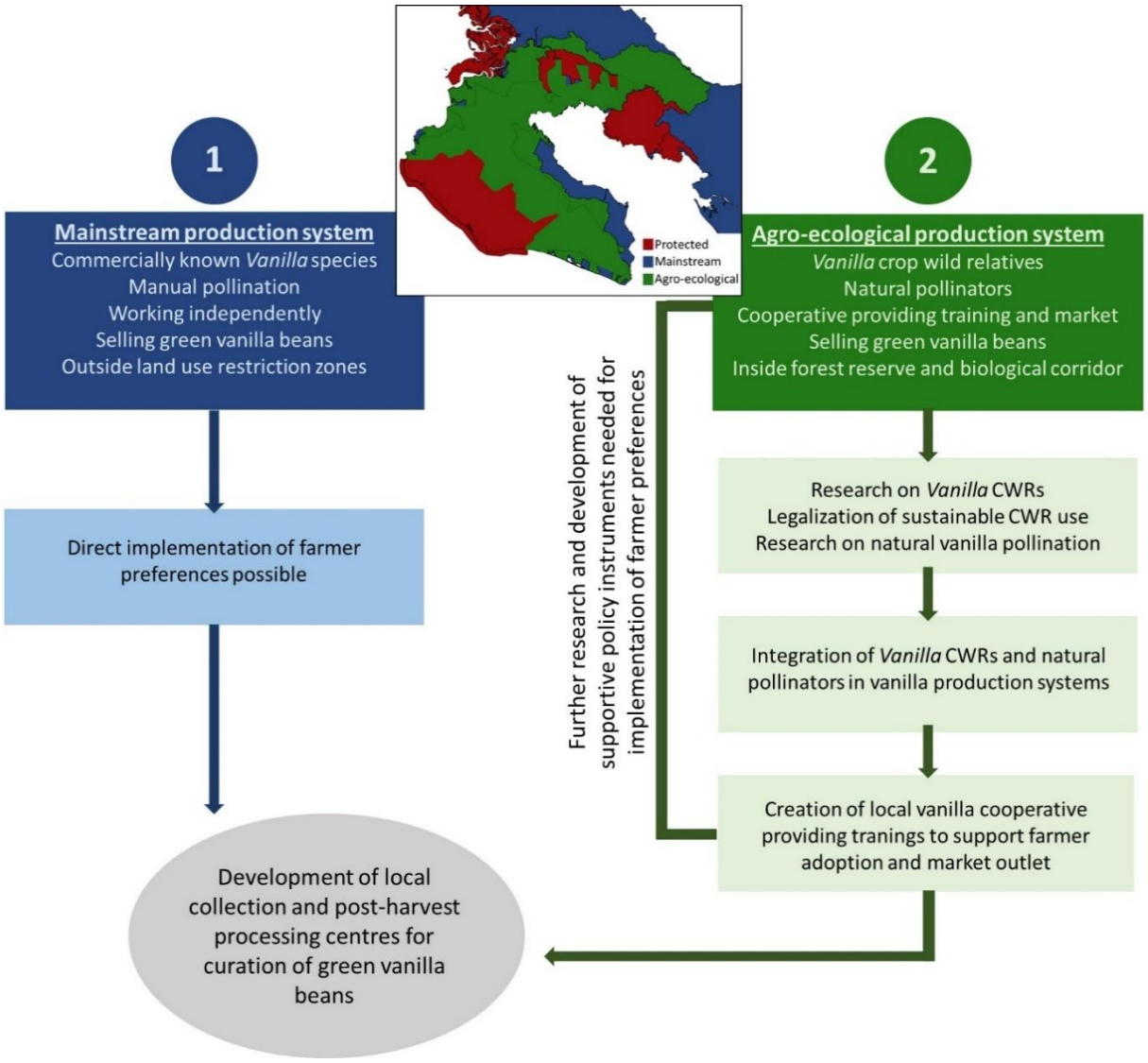

Fig. 6.3. *Schematic overview of the proposed two-tier approach for the implementation of a vanilla cultivation program in a high-priority conservation area such as the Península de Osa, taking into account the distinct differences in preferences among the farmers with interest in vanilla cultivation. The blue and green frames correspond to, respectively, a possible application in the blue and green areas on the map.*

Finally, we find a general preference among the questioned farmers to sell green vanilla beans. This creates scope for investing in local collection and post-harvest processing centres. Such centres could contribute to generating employment, standardizing vanilla bean quality, and assuring a resilient supply chain for high-quality vanilla beans from southern Costa Rica to local and (inter)national markets. On the other hand, an in-depth study on farmer attitudes towards vanilla bean processing is recommended. Meena et al., 2009 and Barua et al., 2017 for example demonstrated that skill-oriented trainings, the organization of farmer groups, and the development of low-cost post-harvest technologies and market-driven extension systems lead to higher farmer participation in post-harvest processing.

6.5.4 Limitations of discrete choice experiments

Since DCEs are based on hypothetical scenarios, there is the possibility for a hypothetical bias (Hensher, 2010), especially since vanilla it not widely cultivated within our study region. It is a major criticism of stated preference data as choices made by individuals in a hypothetical situation might be different from those made in real life situations, due to the fact that their reported choice does not require real action (Hensher, 2010). For example, our surveyed farmers could have made a choice that they, in real life, are not able to implement owing to whatsoever motive. We limited this bias using two ex-ante mitigation tools, previously described as satisfying mitigation techniques (Fifer et al., 2014): (1) respondents were shown an explanatory introduction video with information on the vanilla crop, its management, post-harvest processing, and the current cultivation and market situation, to ensure that they were all equally informed at the start of the survey, and (2) a cheap talk script was used to alert the respondents about the bias and tell them to emphasize on the importance of their answers despite being a hypothetical situation. By openly discussing the potential bias, respondents are more likely to take the bias into account when giving their preferred choice, leading to more accurate results (Cummings and Taylor, 1999).

6.6 CONCLUSIONS

In this study, we aim to reveal the interest and preferences of farmers towards alternative ways of vanilla cultivation using a choice experiment approach and data from 186 farmers in the Península de Osa, Costa Rica. We observe a general positive attitude towards the cultivation of vanilla among the surveyed farmers. We detect significant preference heterogeneity but the majority of farmers with positive attitudes are in favour of (1) agro-ecological systems, with an agroforestry management system involving CWRs and natural pollination, and (2) cooperation in

a cooperative with a production contract. These farmers are mainly located inside the forest reserve or biological corridor, areas assigned to landscape restoration (SINAC, 2018). Hence, more sustainable production systems can be implemented in alignment with the proposed SPASHA approach, whereby horizontal and vertical supply chain coordination might play an important role to facilitate the adoption of the preferred agricultural practices and to ensure a stable market outlet.

We need to note that our study is case-specific and that we should be careful to generalize our findings to the whole farmers' group of the Península de Osa or other regions in Costa Rica and the Neotropics. Nevertheless, our study provides initial insights on farmers' perspectives related to alternative vanilla cultivation and marketing systems, of which many aspects could be studied further for the evaluation of possible implementation programs. Further research on agronomic features of CWRs (e.g. growth, yield and bean quality), their market potential (e.g. aromatic profiles) and the development of supportive institutions and policy instruments is fundamental. Additional studies in similar regions along the Neotropics would be useful to reveal the full potential of agro-ecological vanilla production. Finally, understanding the perceptions of other stakeholders (e.g. consumers, international traders, export companies) along the vanilla value chain will be necessary to accomplish the transformation of the vanilla sector to an efficient, sustainable and resilient system.


7

Chapter 7

Discussion & Conclusions

Photo: Flower of the *Vanilla* crop wild relative *Vanilla trigonocarpa*, growing in the rainforests of our study region in southwest Costa Rica. © Charlotte Watteyn

The vanilla sector faces several problems that are currently preventing a stable vanilla bean supply. The following aspects are key to bring more resilience within both production and marketing: (a) the conservation and use of the available crop genetic pool, (b) the development of robust production systems, and (c) the empowerment of smallholder vanilla farmers. We proposed a strategy that may guarantee both the *in situ* conservation and sustainable cultivation of this high-value spice, defined as the *Vanilla* joint land sparing/sharing (SPASHA) approach. The approach aims at protecting the natural habitat of *Vanilla* species, while developing vanilla cultivation systems that function as biological corridors in areas near these protected habitats; whereby the integration of promising *Vanilla* crop wild relatives (CWRs) as well as fundamental ecological interactions (e.g. pollinators) is encouraged. Considering the current situation within the global vanilla supply chain, empowering smallholder farmers is a complex task, that not only involves a better organization of farmer communities, but also requires a greater transparency and connection among the supply chain members. To provide initial insights into the possible application of this combined approach of vanilla conservation and sustainable cultivation, we explored the distribution patterns and natural pollination mechanisms of four *Vanilla* CWRs (Chapters 2-4), their cultivation and aromatic potential (Chapter 5), and the perceptions of farmers towards innovations in vanilla cultivation and marketing methods (Chapter 6).

7.1 VANILLA CONSERVATION AND SUSTAINABLE CULTIVATION

7.1.1 Theoretical development of a case-specific *Vanilla* SPASHA approach

As highlighted in the introduction, ensuring crop genetic diversity is indispensable within the context of food security (Khoury et al., 2014; Castañeda-Álvarez et al., 2016; Swarup et al., 2021). So far, most efforts have focused on *ex situ* conservation of CWRs (Hajjar & Hodgkin, 2007; Engels & Ebert, 2021). *Ex situ* conservation, however, has several drawbacks, such as finite gene bank storage (Guarino & Lobell, 2011; Castañeda-Álvarez et al., 2016), seed recalcitrance (Bonner, 1990), limited development of new genetic variation (Meilleur & Hodgkin, 2004; Heywood, 2017), and restricted duplication of germplasm accessions (Scarascia-Mugnozza & Perrino, 2022; Engels & Ebert, 2021). Hence, both *ex situ* and *in situ* conservation efforts are recommended. Regarding the vanilla crop, Flanagan & Mosquera-Espinoza (2016) suggested a combined *in situ*, *ex situ* and *circa situm* approach. Building upon their suggestions, we proposed an approach that combines *in situ* conservation with sustainable cultivation of *Vanilla* CWRs within a single territory, and aimed at providing spatially explicit recommendations to implement this case-specific approach within our study region in southwest Costa Rica. We applied species

distribution modelling (SDM) to define suitability areas for the set of modelled *Vanilla* species. Subsequently, the acquired information was translated into a *Vanilla* SPASHA approach for ACOSA that fits within the framework of the National Bio-Corridor Program (SINAC, 2018), as the latter aims at creating productive biological corridors between protected natural areas, thereby providing alternative income generation for the local communities living in these corridors.

A considerable proportion of the modelled species distributions was predicted by the included climate, soil and topographic variables. In general, the mountainous regions of Costa Rica are highly unfavourable for all species, due to the colder temperatures and a less pronounced dry season needed for flowering. Most of the lowland rainforests along the Pacific and the Caribbean coast possess suitable conditions. The drier areas in northwest Costa Rica are only suitable for *V. pompona*. Compared to the other modelled species, *V. pompona* has thicker stems and leaves, which may elicit a higher tolerance towards extreme droughts or other environmental stresses, as previously observed by Jimenez et al. (2017). The lower overall suitability of the other modelled species may be associated with species-specific light requirements or functional plant traits, amongst others. For example, the occurrence of *V. hartii* and *V. odorata* in the understorey of old-growth forests and ridges (Soto Arenas & Dressler, 2010; own survey data) possibly explains their more restricted distribution. Suitability may also depend on the presence of certain biotic interactions, such as those with soil microorganisms, pollinators, and dispersers (Porras-Alfaro and Bayman 2007, Cameron 2011, Flanagan and Mosquera-Espinosa 2016, González-Chávez et al. 2018). Future model inclusion of these specific variables is recommended to fully understand species' habitat suitability and interspecific variability herein.

Although SDM proved useful to identify suitable growing areas for *Vanilla* CWRs, we recognized certain difficulties. The original species set included all *Vanilla* CWRs native to Costa Rica, using occurrence data from online databases, research institutions, and own inventories. Only four species (i.e. *V. hartii*, *V. odorata*, *V. pompona*, V*. trigonocarpa*) presented sufficient occurrence data to construct accurate models. As recognized by Bramel and Frey (2021), vanilla conservation seems to be of low priority for international donors and research institutions, possibly explaining the restricted data availability. Moreover, a recent revision of Karremans et al. (2020) indicated the existence of taxonomic inflation within the *Vanilla* genus. The number of Neotropical species was reduced to 62, meaning that the available occurrence data for a certain species now may allow accurate modelling, or even alter our predicted distributions. A precise taxonomic classification is thus essential to provide valid information on a species' geographical distribution. We also noticed a high level of cautiousness among researchers in sharing data on wild vanilla

locations. The fact that *Vanilla* CWRs belong to the group of species with highest risk of extinction (endangered or critically endangered) (Wegier et al., 2020; Goettsch et al., 2021), most likely explains this prudent behaviour. The latter, however, obstructs the development of reliable niche models and subsequent vanilla conservation programs. Bramel & Frey (2021) emphasized on the formation of a global *Vanilla* research network to enhance our knowledge and the protection of these vulnerable orchids. We highly support this proposition as data could be shared through a confidential data platform managed by this network, allowing the execution of accurate species distribution models while ensuring the protection of wild *Vanilla* populations.

Overall, ACOSA appeared highly suitable for the modelled *Vanilla* species, and suitability maps were combined with regional land use maps to define (a) low- and high-priority conservation areas, and (b) potential cultivation areas. Low-priority conservation was given to protected forested areas with high *Vanilla* species suitability, whereas high-priority was given to forested areas without protection status but high *Vanilla* species suitability. Special emphasis should be given to high-priority areas suitable for species with a more restricted niche distribution (e.g. *V. hartii*), as rare or narrowly distributed species may be the first ones to be negatively affected by environmental alternations (Habel et al., 2020; Loiseau et al., 2020). Both fallow and agricultural lands with high suitability function as potential vanilla cultivation areas. For example, fallow or degraded lands could be converted to reforestation areas including vanilla as a high-value cash crop. Existing agricultural lands, dominantly oil palm and cacao plantations, could be transformed to agroforestry systems with vanilla as an additional crop. Within the theoretical context of the *Vanilla* SPASHA approach, cultivation areas should ideally serve as biological corridors between conservation areas, encouraging the use of promising CWRs and the exchange of ecological interactions such as natural pollination between forests and farmlands.

To provide practical recommendations, besides the theoretical information obtained from the distribution models, regarding the implementation of SPASHA within ACOSA's land use policies, we required further insights on (a) natural *Vanilla* pollination and the viability of pollinator inclusion within a production context, (b) the cultivation and market potential of *Vanilla* CWRs within the abovementioned context (i.e. in reforestation areas or existing agro-systems transformed to agroforestry systems), and (c) the perspectives of farmers towards this approach.

7.1.2 Natural pollinators and their potential integration within vanilla cultivation

Pollination is crucial for the reproduction and long-term survival of plant species, and occurs via autonomous self-pollination or is mediated by (a)biotic vectors. The latter are responsible for the removal and subsequent allocation of pollen on the stigma of the same (autogamy) or a different (allogamy) flower (Ollerton, 2021). Allogamy or cross-pollination assures gene flow, thereby increasing the genetic diversity within and among plant populations (Dodd et al., 1999; Ollerton, 2021). Regarding the vanilla crop, autonomous self-pollination is not known to occur, and it is generally seen as pollinator-dependent. However, little is known about its natural pollinators. Farmers apply manual pollination as it guarantees satisfying yields in case of pollinator limitations (Wurz et al., 2021). Despite its benefits, manual pollination is labour-intensive and reduces gene flow, as pollen is placed on the stigma of the same flower (Bory et al., 2008). Integrating natural pollinators into the pollination management scheme could greatly enhance the agro-ecological value of corresponding vanilla cultivation systems and potentially decrease labour costs. Moreover, a better understanding of these fundamental ecological interactions allows us to fine-tune *Vanilla* conservation plans. The limited knowledge on *Vanilla* pollination brought us back to the foundations of pollination ecology, and within the timeframe of this PhD thesis, we obtained considerable insights into the pollination mechanisms of two of the above modelled species: *V. pompona* and *V. hartii* (Fig. 7.1) (Chapters 3-4).

Vanilla pompona is pollinated by male *Eulaema cingulata*, and our morphological assessment indicates that other, similar *Eulaema* species could also act as pollinators – which conforms with previous reports (*Eulaema nigrita*, Ackerman 1983a; *Eulaema meriana*, Lubinsky et al., 2006 and Householder et al., 2010). Combining the results from the behavioural observations and phytochemical studies, we suggest a dual pollinator attraction mechanism of male *Eulaema cingulata* to *V. pompona* flowers, employing floral fragrance rewards as the initial attractant and food deception to induce pollen removal and deposition. In contrast, *V. hartii* is a nectar-rewarding species, proven by the presence of extractable nectar dominated by sucrose. *Vanilla hartii* is pollinated by a range of *Euglossa* species that search for nectar in the labellum, thereby successfully removing the pollen in case of a morphological fit. Based on these two species, predictions can now be formulated for other Euglossini-pollinated Neotropical *Vanilla* species. For example, the supposedly food-deceptive *V. dubia* (Pansarin & Pansarin, 2014) seems to rather display a dual mechanism similar to *V. pompona*, as male *Euglossa* demonstrated fragrance collection behaviour on the sepals and tepals, but were lured into the labellum by means of food deception. Moreover, *Epicharis* (Apidae: Centridini), the assumed pollinator of *V. edwalii*

belonging to a bee tribe different from Euglossini, also seems to be attracted to the floral fragrances emitted by the nectarless flowers of *V. edwalii* (Pansarin et al., 2014). Hence, various *Vanilla* species may have developed a dual pollinator attraction mechanism that involves a combination of floral fragrances and food deception.

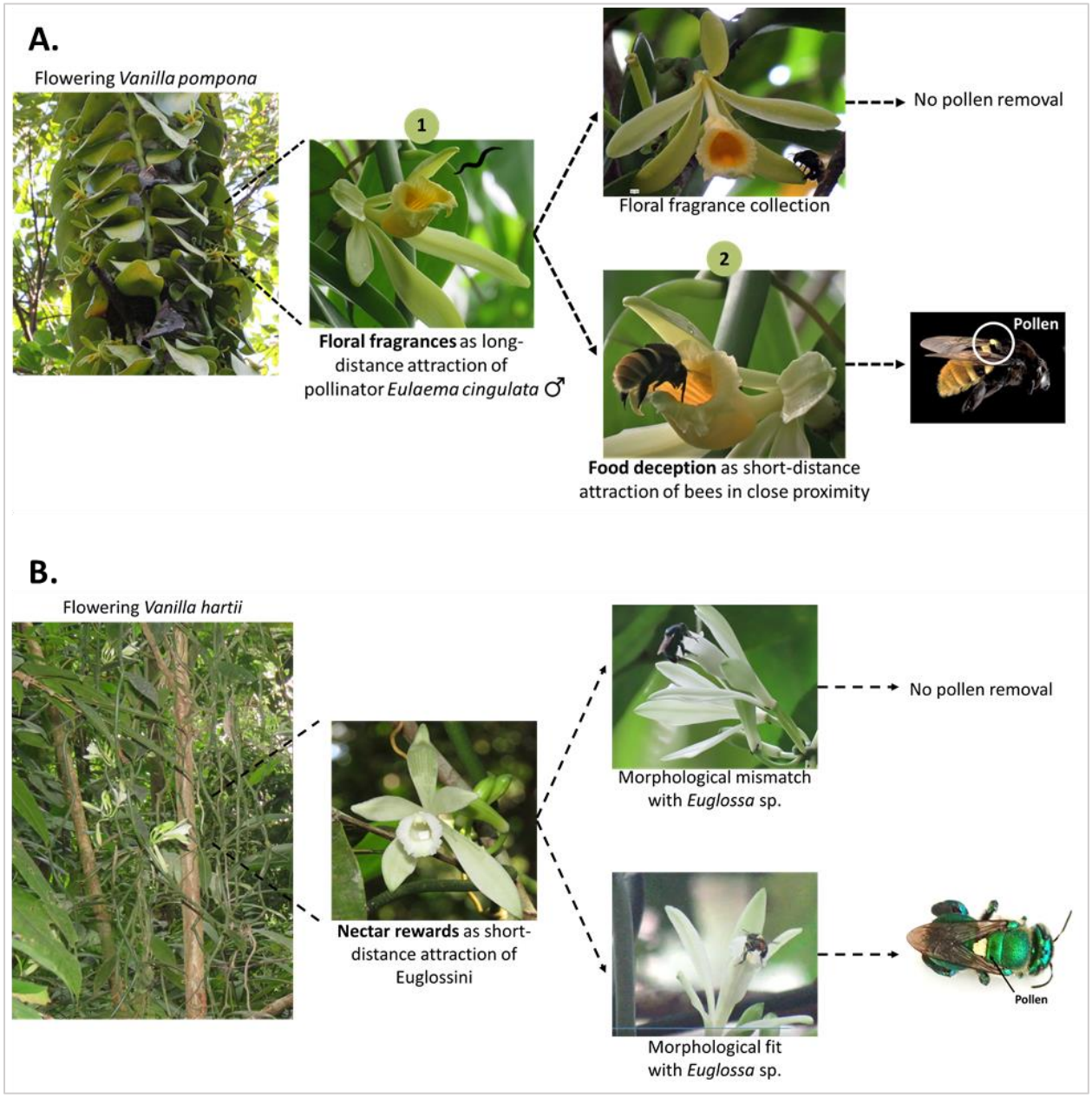

Fig. 7.1. *Schematic representation of (A) the dual pollinator attraction mechanism in V. pompona and (B) the nectar-rewarding pollination mechanism in V. hartii.*

Vanilla pompona had a lower reproductive success compared to *V. hartii*, as expected being a food-deceptive and nectar-rewarding species, respectively. However, the reproductive success of these two species varied among sampling sites, which may be due to spatial or temporal variability in Euglossini bee presence and abundance. In an additional study of Van der Schueren et al. (2020), we observed a significant effect of habitat disturbance, forest cover, and weather on Euglossini species richness and abundance within our study area. The observed weather effects on Euglossini diversity, which are conform with previous studies reporting an increase in Euglossini activity during the dry and early wet season (Ackerman, 1983; Melo et al., 2009), possibly explain the *Vanilla* flowering peak during the dry season (December-April for ACOSA), as to coincide with highest bee activity. Habitat disturbance (i.e. percentage of disturbed habitat present in a 1 km radius around sampling sites) negatively affected Euglossini diversity, while the latter increased with forest cover (i.e. percentage of old-growth forest present in a 1 km radius around sampling sites) – observations that coincide with previous studies of do Knoll & Penatti (2012) and Botsch et al. (2017). More precisely, habitat disturbance and reduced forest cover negatively affected the abundance of small and medium *Euglossa*. Larger *Euglossa* and *Eulaema* did not seem to respond to habitat disturbance nor forest cover, which conforms with records of *Eulaema* navigating across disturbed areas (Dressler, 1986; Milet-Pinheiro & Schlindwein, 2005).

Based on the above, we recognize the importance of preserving intact old-growth forest patches surrounding wild *Vanilla* populations, especially for the species pollinated by smaller *Euglossa* (e.g. *V. hartii*). In this context, the wider distribution range of *V. pompona* may be linked to the higher resilience of its pollinators against habitat disturbance. For example, *Eulaema* bees navigating across a fragmented landscape, are able to transfer pollen from one *V. pompona* population to another, thereby increasing gene exchange and thus adaptation capability (Fig. 7.2). On the other hand, *V. hartii* populations are more restricted to old-growth forests, and seemingly are its pollinators. Hence, restricted pollen transfer among *V. hartii* populations decreases genetic variability, which in turn leads to more restricted adaptation capabilities. Additionally, various orchid pollination studies (e.g. Cozzolino et al., 2005; Jersáková et al., 2006; Stípková et al., 2020) suggest that deception evolved as a mechanism to avoid inbreeding depression, as the lack of rewards reduces the number of pollinator visits per inflorescence. It may elucidate the evolutionary success of deceptive mechanisms among the *Vanilla* species (i.e. the isolated position of the nectar-rewarding *V. hartii* as a sister to most of the other, seemingly deceptive, members of *Vanilla* sect. *Xanata* (Bouetard et al. 2010; Soto Arenas & Dressler 2010; Azofeifa-Bolaños et al. 2017)), and possibly provides an additional explanation for the higher adaptation capacity of *V. pompona* compared to *V. hartii*. These hypotheses should, however, be further

explored via phylogenetic studies that link the genetic variability within and among populations with corresponding pollinators and pollination mechanisms.

Although the knowledge gap on *Vanilla* reproduction biology remains large, we described the pollination mechanisms of two *Vanilla* CWRs and observed the display of distinct pollination mechanisms across the allogamous species (Table 4.1). The latter are pollinated almost exclusively by members of the Apidae family (the exception being *V. siamensis*, which is pollinated by Halictidae), and there possibly exists phylogenetic and biogeographic specialization. For example, the allogamous species of *Vanilla* sect. *Xanata* – the fragrant *Vanilla* CWRs that are endemic to the Neotropics – seem to depend exclusively on euglossine bees for pollination, a trend originally identified by Soto Arenas (2003). We recognize, however, one main drawback for successful integration of pollinators within a production context: a low natural fruit set. Orchids with food-deceptive mechanisms are known for their low reproductive success (Scaccabarozzi et al., 2020), and although the nectar-rewarding *V. hartii* has a higher fruit set compared to its congeneric deceptive species, it is still not favourable in terms of production. For agricultural purposes, optimizing manual pollination techniques or developing autogamous cultivars may deliver more productive outcomes, as previously recognized by Flanagan & Mosquera-Espinoza (2016). Within the framework of the *Vanilla* SPASHA approach, however, we highly promote a vanilla cultivation landscape that consists of protected forests (i.e. *Vanilla* and Euglossini refuges) surrounded by Euglossini-friendly vanilla cultivation areas. The latter being agroforestry systems boasting more similarity to a forest ecosystem than a vanilla monoculture, and thereby serving as corridors between forest patches, and potentially stimulating the exchange of pollination services between forest and farmland. A study in Mexico, for example, demonstrates that, in contrast to coffee monocultures, the Euglossini community of coffee agroforestry systems was similar to that of adjacent forest patches (Briggs et al., 2013). Certification of vanilla beans, although manually pollinated, produced in these landscapes could have a higher market value, and beans resulting from natural pollination could even be sold at specialty markets. This in turn encourages the development of such agro-ecological landscapes within areas such as ACOSA, where conservation policies are restricting the development of certain economic practices.

Combining pollinator behaviour and phytochemical studies proved useful in revealing *Vanilla* pollination mechanisms. Yet, these studies are challenging and time-consuming considering (a) the necessity to precisely track a species' flowering period, (b) natural populations being merely found in remote sites with difficult access, and (c) the many field hours required to observe pollination events, identify corresponding pollinators and pollination mechanisms, and quantify

reproductive success. We first tried a less time-consuming observation method involving automatic cameras (Steen, 2017), but these were not successful as the camera sensitivity decreases with increasing distance between camera and flowers (vanilla flowers are usually found at least a couple of meters aboveground, and the terrain not always allows sufficient proximity). Hence, we followed standard field observation methods as described in Yamaji & Ohsawa (2016). Since we assume that the allogamous species belonging to *Vanilla* sect. *Xanata* are pollinated by euglossine bees, several Euglossini species could be collected at baits and alternatively placed together with flowering *Vanilla* species in an enclosed flying cage. This experimental set-up could help to optimize vanilla pollination studies.

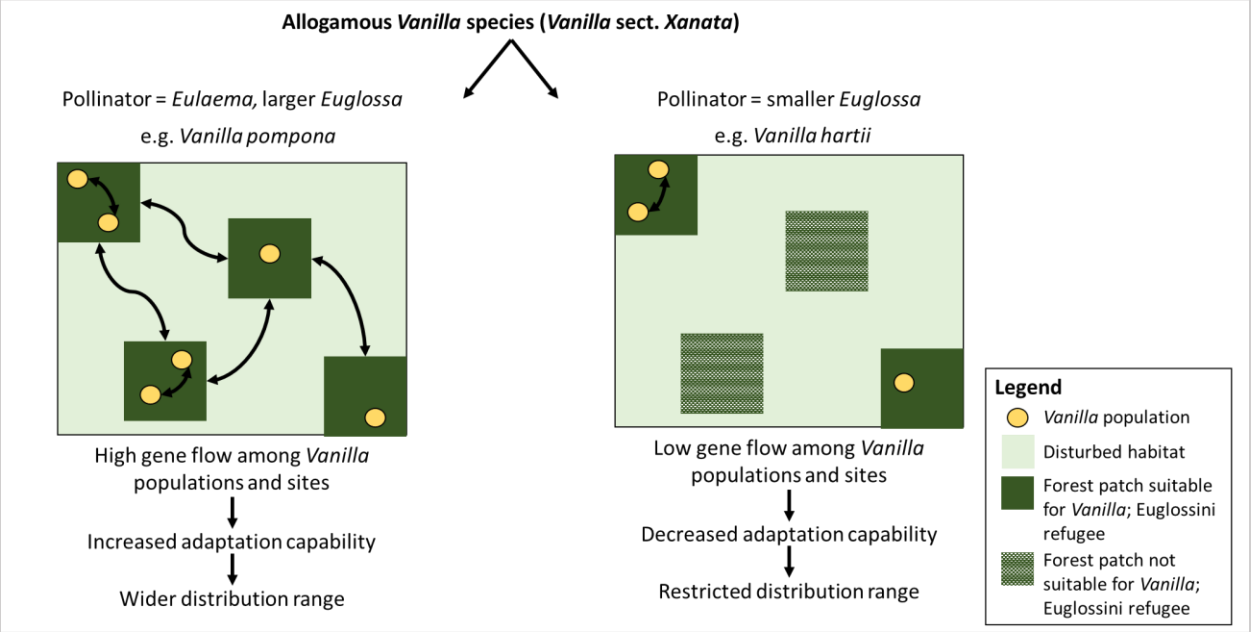

Fig. 7.2. *Schematic representation of the formulated hypothesis that links the observed species distribution patterns of V. pompona and V. hartii with the habitat requirements of their pollinators.*

7.1.3 Cultivation potential of *Vanilla* CWRs within context of SPASHA

CWRs are vital for the agricultural sector as they may contain a set of traits useful for crop improvement, and thereby enhance the resilience of food production systems (Dempewolf et al., 2017). *Vanilla* CWRs may contribute to the mitigation of the observed production problems within the vanilla sector, as described in the introduction and highlighted by, amonst others, Flanagan et al. (2016) and Bramel & Frey (2021). Recent genotypic characterization reported considerable diversity among and within *Vanilla* species, which may be of value for crop improvement and breeding (Hu et al., 2019; Hasing et al., 2020; Chambers et al., 2021). Studies exploring the potential of *Vanilla* CWRs within certain production settings are thus highly needed. We carried

out a field experiment to provide initial insights on the plant performance of six species – four CWRs, the crop species *V. planifolia* and a commercially-used hybrid ((*V. planifolia* x *V. pompona*) x *V. planifolia*) – and in two land use systems. In the landscape context of our case-specific *Vanilla* SPASHA approach, *Vanilla* CWRs could be cultivated (a) on reforested fallow or degraded lands, or (b) within existing agro-systems; both surrounded by forests holding wild populations of corresponding CWRs. We selected naturally regenerated secondary forests (SF) due to the unavailability of reforested areas, and cacao plantations (CP) as a type of existing agro-system.

The overall health performance of the hybrid and the CWRs was higher compared to *V. planifolia*, with the vigorous hybrid and *V. pompona* outperforming the other CWRs. *Vanilla pompona* being a stress-resistant species (Koyyappurath et al., 2015), and the hybrid a crossing between *V. pompona* and *V. planifolia* (Grisoni et al., 2021), most likely explains this trend. On the other hand, both the hybrid and *V. planifolia* outperformed the CWRs in terms of growth, possibly due to previous selection of fast-growing *V. planifolia* phenotypes (Bory et al., 2007; Lubinsky et al., 2011). The interspecific differences are likely associated with species-specific functional plant traits, which were not measured in our study. Koyyappurath et al. (2015), for example, correlated the higher resistance of *V. pompona* with its lignified hypodermal cell walls and phenolic root exudates. Moreover, species-specific performance depended on light availability and land use system. *Vanilla hartii* and *V. odorata* performed better with limited light intensity, which conforms with their natural occurrence in the understory of old-growth forests and on ridges (Soto Arenas & Dressler, 2010; Watteyn et al., 2020), whereas the other species performed better with increasing light intensity. We, however, measured onsite light availability. To fully understand species-specific responses to light, a broader light spectrum should be examined – as previously carried out for *V. planifolia* (Puthur, 2005; Díez et al., 2017). Compared to the other species, *V. planifolia* performed less in SF compared to CP, which may be due to a combination of both reduced light conditions and other, non-measured variability among the two land use systems.

The plant performance of all *Vanilla* species was positively affected by the abundance of disease-suppressive and growth-promoting soil bacterial phyla. This underlines the existence of specific interactions between vanilla plants and soil microbiota – as previously highlighted in a number of studies (e.g. Porras-Alfaro & Bayman, 2007; Bayman et al., 2011; Dearnaley et al., 2012; Flanagan & Mosquera-Espinoza, 2016; Johnson et al., 2021). Furthermore, the observed effects of soil organic matter, nutrients, and pH on vanilla plant performance were conform with past studies of La et al. (1998), Osorio et al. (2014), Chen et al. (2015) and Xiong et al. (2017), who stressed on the importance of a proper soil management for the vanilla crop (e.g. pH between 6-

7, high organic matter content and P levels). Additionally, we were the first to correlate onsite tutor tree functional traits with vanilla plant performance, and observed a positive effect of high SLA values and low leaf C:N ratio. The selection of adequate trees may thus be focal point in the design of vanilla agroforestry systems, as has been highlighted for cacao plantations (Sauvadet et al., 2019). A better understanding of potential feedback loops between soil properties, soil microbiota, and functional tree traits, jointly affecting vanilla plant performance, could greatly enhance the interpretation of the above observed trends, and enable the design of resilient vanilla cultivation systems.

The display of earliness in *V. hartii*, *V. odorata* and *V. pompona*, and the fact that *V. hartii* and *V. odorata* have two flowering periods per year, one in the dry and one in the wet season (Soto Arenas & Dressler, 2010; own survey data), are useful traits in terms of production. Yet, further examination is required to define the underlying causes and continuity of these observed patterns, and to quantify annual yields at species level. Unfortunately, within the two-year timeframe of our study, we could not collect the above data as species just (earliness) or did not yet started to flower. Although data on vanilla bean production is still lacking, we observe potential for the inclusion of the hybrid and *V. odorata* in the food industry, as both showed attractive levels of vanillin and other compounds (pHB and anisyl) – an aromatic profile comparable to the crop species *V. planifolia*. The high levels of anisyl compounds in *V. hartii* and *V. pompona* indicate their potential for specialty markets – as previously indicated by Maruenda et al. (2013) and Peréz Silva et al. (2021). Although *V. helleri* contained all examined aromatic compounds, they were present in small amounts, and the same holds for *V. trigonocarpa*. Yet, chromatographs of these species showed a variety of other peaks, which could represent compounds (e.g. fatty acids, unidentified aromatic compounds) of interest for the industry and require further examination.

Based on the above, we see potential for the integration of *Vanilla* CWRs within the defined production context, as our initial results from the experimental field sites demonstrated (a) a good overall plant performance, (b) early flowering phenotypes, and (c) interesting aromatic profiles. Cultivation areas should present adequate soil microbiota, tutor trees, physicochemical soil characteristics, and species-specific light intensities. Hence, successful vanilla integration within existing agro-systems such as the selected cacao plantations may require a spatial transformation of the current designs. We already noticed a certain level of competition between the cacao trees and the incorporated vanilla tutor trees, which may negatively affect long-term crop management. Moreover, the observed *Vanilla* species-specific light preferences together with cacao trees' light requirements, will certainly entail a precise spatial design and corresponding management (e.g.

pruning). Since no reforestation areas were available, we selected naturally regenerated secondary forests, yet, observed a number of drawbacks: randomly spread tutor trees, typical for naturally regenerated forests (Zhang et al., 2020), and the lack of suitable tutor tree branching patterns. These limitations prevent a proper guidance of vanilla vines from one tree to another (i.e. vanilla curtains) or within the same tree (i.e. up- and downwards coiling). Correct vine management is vital to facilitate manual pollination and harvest, and to ensure sufficient ventilation (Havkin-Frenkel & Boulanger, 2018). Besides, the suggestion of cultivating crops within existing forests has received quite some criticism in the past (e.g. Aerts et al., 2011), as farming activities may negatively affect forest biodiversity and associated ecosystem services. We therefore recommend to implement so-called vanilla forests on fallow or degraded lands, hereby selecting and distributing a mix of canopy trees according to *Vanilla* species-specific light preferences. Vanilla tutor trees should ideally be incorporated in the understory. Moreover, they should enable a proper vine management, and possess favourable functional traits such as high SLA and leaf N content, low branching patterns, and non-peeling barks (e.g. *Erythrina*, *Diphysa*, *Gliricidia*).

Based on the results of our pollination studies (Chapters 3-4) and field experiment (Chapter 5), we propose a possible *Vanilla* SPASHA design for our study region ACOSA (Fig. 7.3) as well as a schematic overview of the combined results from Chapters 2 to 5 (Fig. 7.4). The designs encourage the implementation of agro-ecological cultivation systems that mimic the conditions of vanilla's natural habitat, something that is far more challenging to establish in monocultures or artificial greenhouses. Yet, it vastly needs further evaluation in terms of feasibility, as we were not able to collect data on production, labour, and revenues within the time frame of this PhD research. To provide further practical recommendations for the implementation of this case-specific *Vanilla* SPASHA approach, the following aspects related to management, production, as well as biodiversity should be examined, and ideally be compared with intensively managed vanilla monocultures:

- Optimal light conditions and associated spatial distribution of the different *Vanilla* species as well as other included crops required to achieve maximal production.
- Calculations of yield, costs and revenues associated with the cultivation of the different *Vanilla* species as well as other included crops. Focusing on the vanilla crop, this would include:
    - Flowering patterns: flowering periods, number of inflorescences and flowers.
    - Fruit development: percentage of aborting or deformed fruits, fruit capacity per plant.

    - Labour costs related to manual pollination, harvest, and management, taking into account the possibility of multiple flowering periods per year of certain *Vanilla* species as well as the potential lack of flowering synchronization among the species.
    - Market prices: estimated market value of beans corresponding to the different *Vanilla* species.
- Biodiversity indices that evaluate the functionality of the proposed agroforestry systems as biological corridors within the SPASHA context.

Besides the need to further evaluate the feasibility of this approach, we need to recognize the certain threat for admixture between the wild *Vanilla* gene pool and vanilla cultivars. Cultivating a mix of *Vanilla* CWRs and commercially-used species (e.g. hybrid used in this study and the crop species *V. planifolia*) in the surroundings of natural forests holding wild *Vanilla* populations may lead to hybridization and introgression – as has been observed with coffee (Aerts et al., 2012). Potential risks could, however, be minimized by only allowing the use of local species in the cultivation areas directly neighbouring natural forests, thereby serving as a buffer with the cultivation areas that may include improved cultivars. Genotyping the populations found in ACOSA and its surroundings could enhance our knowledge on the existing genetic diversity present within the wild *Vanilla* gene pool, and improve the development of appropriate *in situ* and *ex situ* conservation as well as cultivation schemes.

SPASHA is a combination of *in situ Vanilla* conservation and sustainable cultivation, i.e. the development of agroforestry systems that incorporate promising *Vanilla* CWRs and serve as biological corridors between protected forest patches holding wild *Vanilla* populations. Yet, as mentioned before, a combination of *in situ* and *ex situ* conservation is recommended. The exploration of *ex situ* conservation actions was out of the scope of this thesis, but their importance to preserve vanilla genetic resources as a complement to *in situ* and *circa situm* conservation strategies (Flanagan & Mosquera-Espinoza, 2016; Menchaca & Lozano, 2018; Bramel & Frey, 2021) should be highlighted. Flanagan & Mosquera-Espinoza (2016), for example, described specific actions for *ex situ Vanilla* conservation, including the establishment of (a) living collections of existing genotypes, (b) cryopreserved seed banks, (c) *in vitro* germplasm collections maximizing genetic diversity, (d) research programs that develop protocols for *ex vitro* acclimatization for both population restoration and cultivation, and (e) mycorrhizal germplasm collections to facilitate *ex situ* propagation as well as cultivation and population restoration. *In vitro* plants of prioritized genotypes could accordingly be multiplied and certified as planting material to be distributed among farmers. This may decrease the risk of introgression associated with the

incessant regeneration of identical (clonal) material (Divakaran & Babu, 2009). Furthermore, *ex situ* collections provide material for the continuous evaluation of genotype-specific plant performance (e.g. via controlled experiments testing abiotic and biotic stress resistance or productivity), and the subsequent gene identification of specific traits interesting for crop improvement (i.e. disease-resistance, organoleptic features, fast growth, early flowering). Yet, to obtain the necessary information regarding available genotypes, and to evaluate their potential for cultivation, large-scale genotyping-by-sequencing programs (e.g. using SNP markers) (Metzker et al., 2010; Fabre et al., 2022) are required, and this in both wild *Vanilla* populations as well as existing gene banks and botanical gardens. From here, a baseline can be provided to prioritize *in situ* conservation areas, to assess losses of certain varieties, and to reintroduce lost varieties that have been conserved *ex situ* into production systems (Halewood et al., 2018).

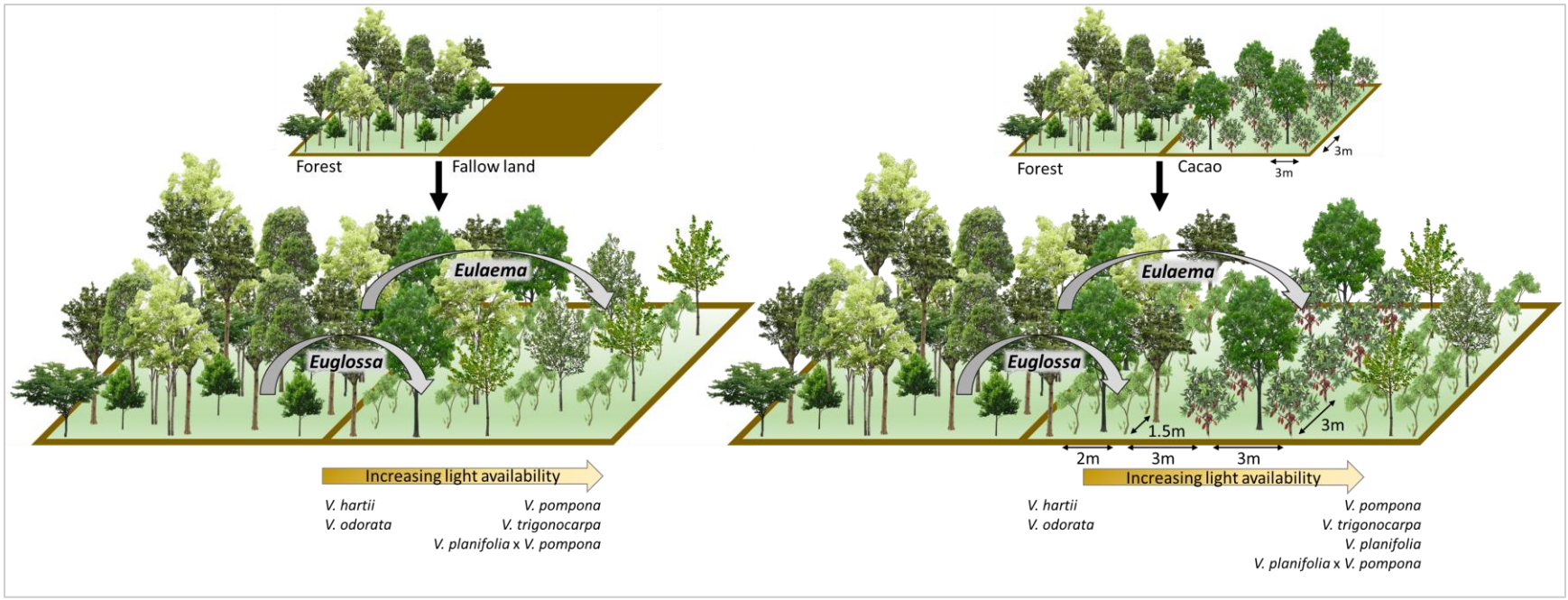

Fig. 7.3. Possible design for the *Vanilla* SPASHA approach that (a) promotes the presence of forest patches (*Vanilla* and Euglossini refuges) surrounding Euglossini-friendly vanilla cultivation areas to enhance possible pollinator exchange between forest and farmland, and (b) takes into account the observed *Vanilla* species-specific preferences that allow optimal vanilla plant performance within the cultivation areas. For example, *V. hartii* and *V. odorata* favour limited light availability, hence, a habitat resembling old-growth forests, as do their pollinators (smaller *Euglossa*). The other *Vanilla* species require higher light intensities and thus tutor trees with less dense canopies and a larger spatial distribution. Since the identified pollinators of these species (*Eulaema* and larger *Euglossa*) are able to navigate across areas with a certain level of disturbance, they should be able to traverse the less densely covered vanilla cultivation areas. The implementation could be carried out on a fallow or degraded land (left) or on existing agricultural lands such as a cacao plantation (right). The latter will require a spatial transformation of current designs (3 m x 3 m).

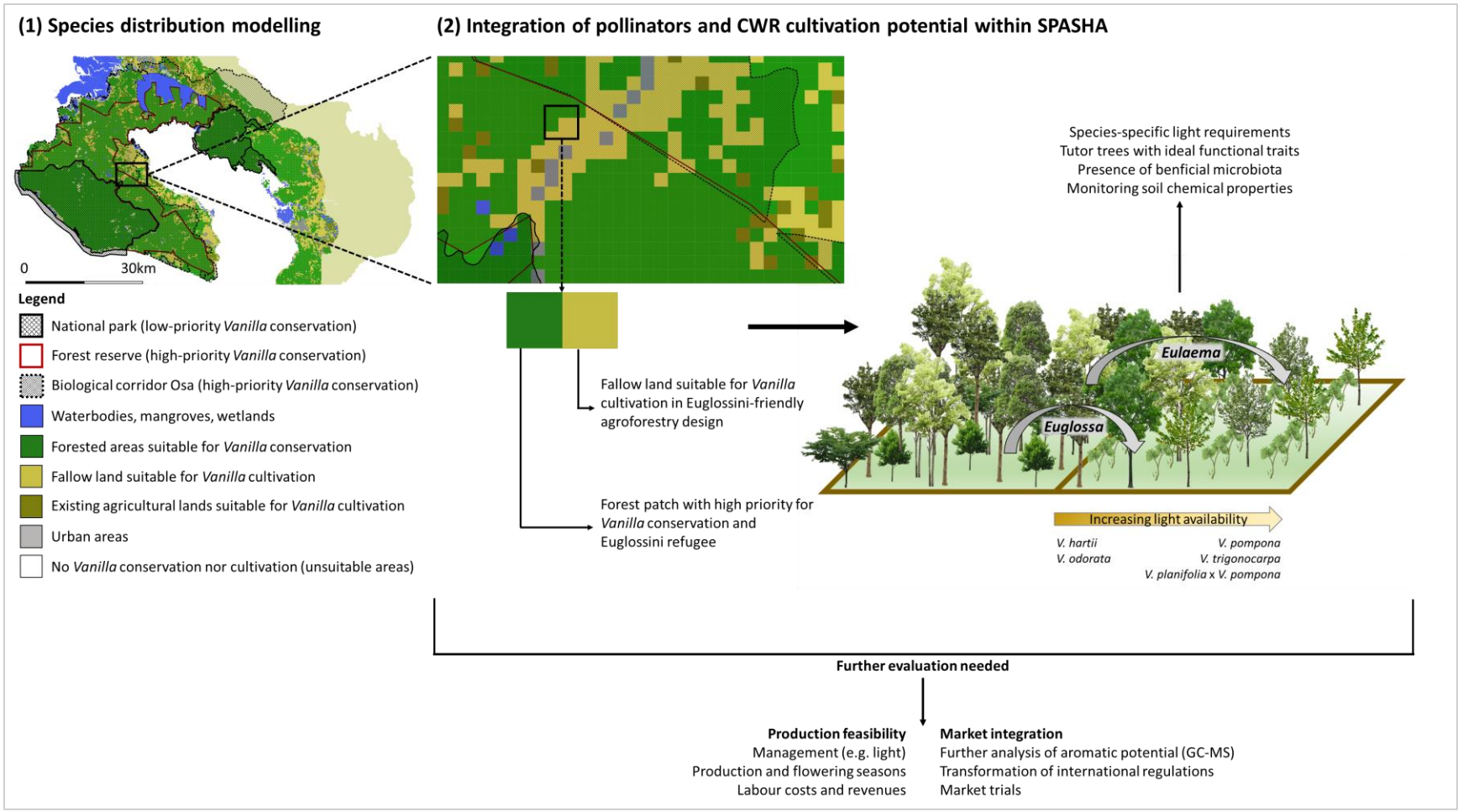

Fig. 7.4. Schematic overview of potential implementation of the *Vanilla* SPASHA approach within our study region ACOSA, based on the results of the SDM (Chapter 2), pollination studies (Chapters 3-4), and the field experiment (Chapter 5).

7.1.4 Perceptions on innovations within the vanilla supply chain

In order to successfully implement new strategies within food production and trade systems, the perspectives of all chain members should be well-understood. We aimed at examining the interest and preferences of farmers living within ACOSA (Chapter 6) towards innovations in the vanilla sector. A short survey followed by a discrete choice experiment (DCE) was performed. A DCE is a quantitative technique used in the absence of revealed preference data (Hanley et al., 1998; Mangham et al., 2009; Van den Broeck et al., 2017), which is applicable in our case as we explored hypothetical scenarios.

The farmer survey indicated that most of the farmers (67.2%) living in ACOSA perceive vanilla cultivation as an improvement upon their current situation. One of the main outcomes was that almost half of the questioned farmers (44.8%) demonstrated a strong preference towards the cultivation of new *Vanilla* varieties (CWRs) within agroforestry systems, the integration of natural pollinators, and the formation of a cooperative providing training and market outlet. As previously shown by Borbolla-Perez et al. (2017), vanilla cooperatives play an important role in informing smallholder farmers about genetic improvements, sustainable farming practices, quality standards, and market outlet, and several other studies (e.g. Kolade and Harpham, 2014; Ahmed and Mesfin, 2017; Manda et al., 2020; Olagunju et al., 2021) emphasized on the important role of cooperatives in providing technical assistance, especially for farmers with less market access or a lack of market awareness. Hence, cooperatives will probably play a crucial role in farmers' adoption of sustainable vanilla cultivation methods. The attitude of abovementioned farmers is noteworthy considering their more remote location within the landscape context of ACOSA, i.e. areas with a stricter land use policy and bordering protected areas. As highlighted, the SPASHA approach has a clear added value within these areas, and seems to be in alignment with the preferences observed among the residing farmers. The remaining farmers showing interest towards the vanilla crop (24.4 %) prefer to use commercial vanilla varieties, apply manual pollination, and work independently. The fact that these farmers are living merely closer to the villages and outside land use restriction areas may explain their preferences, as the implementation of agricultural activities has less restrictions and access to processing units or buyers is probably more straightforward.

In an additional study led by Blaakman et al. (2022), the perceptions of Belgian and Dutch consumers as well as businesses (restaurants and food processors) towards alternative vanilla production (new species with different shapes and aroma, sustainable agroforestry systems) and marketing (origen, certification, price) systems were evaluated. Former studies (e.g. Ricci et al.,

2018; Aschemann-Witzel et al., 2019; Panda et al., 2020) indicated that consumers are increasingly aware of the problems related to current food production and trade systems, leading to a growing demand for products derived from sustainable production systems and with fair revenue partition. Various businesses (e.g. Verstegen Spices & Sauces, Callebaut, Ben & Jerry's) pledged to invest in the development of strategies that guarantee a sustainable supply of high-quality vanilla products and better living conditions for smallholder farmers (CBI, 2018; personal communication and websites of corresponding businesses[1]). Blaakman et al. (2022) indicated that the questioned businesses (i.e. B2B) showed preferences towards certified vanilla beans with organoleptic features different than the standard aroma, and vanilla cultivation in agroforestry systems within the centre of origin (i.e. Neotropics). They demonstrated disfavour towards short and thin vanilla beans and greenhouse monocultures. This trend may be due to B2B respondents being (a) more aware of the problematic situation within the vanilla sector, (b) well-informed about the possible advantages of agroforestry systems, and (c) more interested in new and diverse products that may broaden market outlets. On the contrary, no specific preferences were observed among the end-consumers (B2C), possibly due to vanilla beans being rather sporadically purchased by the common consumer, resulting in unpronounced statements. In conclusion, B2B respondents showed interest towards the proposed innovations in vanilla production and marketing, i.e. new *Vanilla* varieties with different aromas and produced in agroforestry systems in the centre of origin. Hence, involvement of this market segment will probably be key to ensure a successful development of innovative vanilla supply chains.

Based on the observed preferences, we developed a potential supply chain scheme for ACOSA (Fig. 7.5). Farmers interested in the application of agro-ecological practices involving *Vanilla* CWRs, natural pollinators, and agroforestry systems, prefer to be part of a cooperative providing training and market outlet. This coordination among farmers through cooperatives, as well as between farmers and businesses that highly value sustainable and innovative production and marketing systems, could greatly enhance rural income mobility and poverty alleviation, thereby contributing to several of the SDGs mentioned at the beginning of this thesis, such as zero hunger, responsible consumption and production, climate action, and life on land. ACOSA is one of the most biodiverse but poorest regions of Costa Rica (INEC, 2021), and the assignation of protected areas, such as national parks and forest reserves, comes along with certain land use restrictions. As such, approaches such as the Vanilla SPASHA approach could improve the economic situation of smallholder farmers living within these areas. Yet, besides an evaluation of production feasibility (see 7.1.3.), additional insights into international market regulations allowing the integration of new *Vanilla* varieties is highly required.

[1]https://www.verstegen.co.uk/sustainability/agroforestry; https://www.barry-callebaut.com/en/manufacturers/sustainability-in-action/vanilla; https://www.benjerry.com/values/issues-we-care-about/fairtrade.

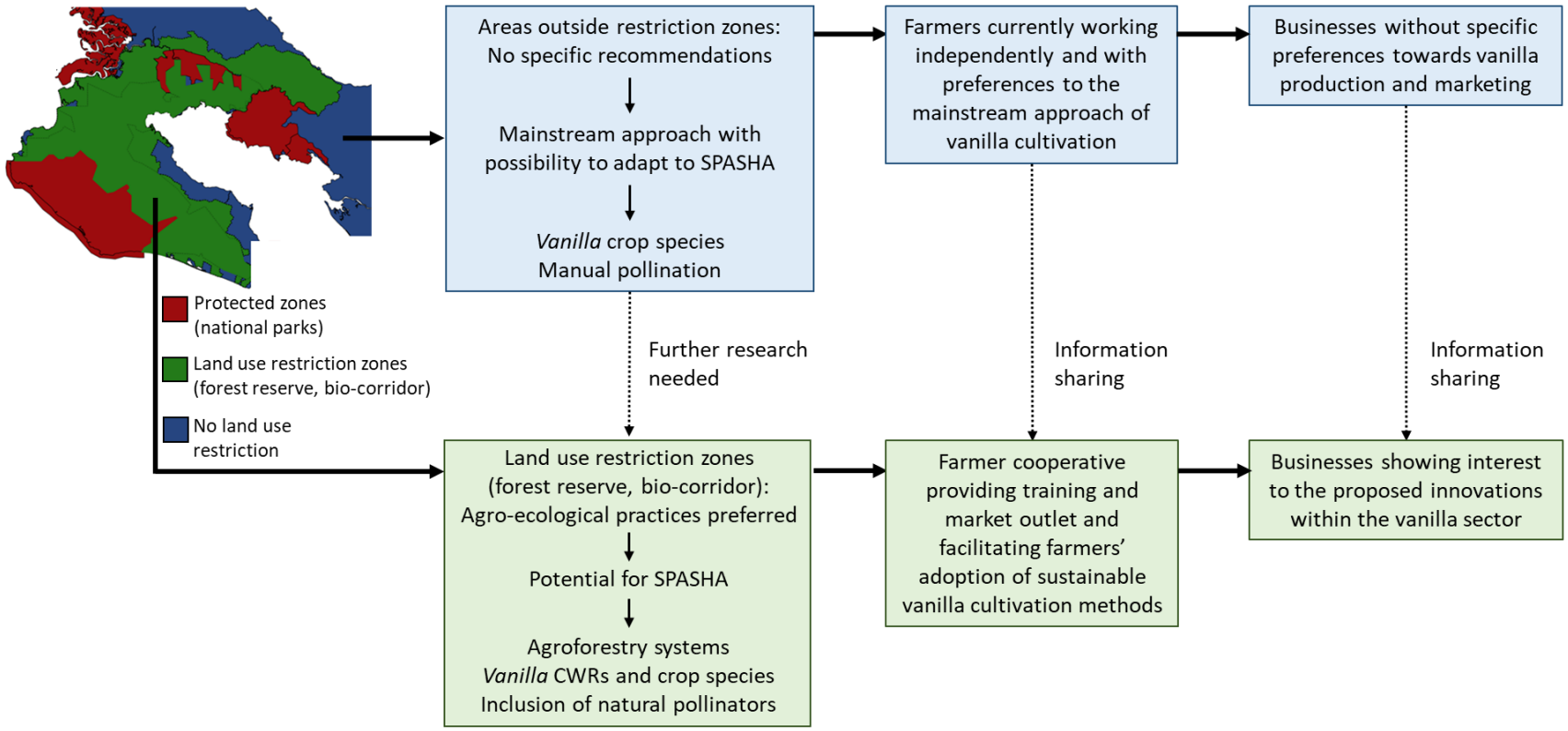

Fig. 7.5. Potential supply chain scheme related to the observed preferences among farmers and businesses. The blue and green frames correspond to, respectively, a possible application in the blue and green areas on the map.

7.1.5 From theory to practice

Based on the results of this thesis and in collaboration with farmers living in ACOSA, a plan to implement sustainable vanilla cultivation systems and establish a transparent supply chain is currently in development. So far, 23 farmers committed themselves to diversify 0.5 to 1 ha of their farmlands with vanilla and other crop species. During several workshops, the farmer group developed and discussed several agroforestry designs in collaboration with PRETATERRA and Verstegen Spices & Sauces B.V. The latter is a European spice company recognized for its sustainable practices (European Business Award for the Environment, 2021) and with interest to purchase vanilla from agroforestry systems implemented by this farmer group. Due to current restrictions on commercial *Vanilla* CWR use and the low fruit set resulting from natural pollination, the following decisions were made:

- Integration of the hybrid ((*Vanilla planifolia* x *Vanilla pompona*) x *Vanilla planifolia*) into diverse agroforestry systems. Instead of the widespread crop species *V. planifolia*, farmers chose the hybrid as it demonstrated an excellent overall plant performance and aromatic potential (Chapter 5).
- Application of manual pollination to ensure satisfying yields and thus revenues.
- Development of Euglossini-friendly vanilla cultivation systems, as *Euglossa* and *Eulaema* species were identified as the pollinators of fragrant *Vanilla* CWRs (Chapters 3-4); hereby encouraging the exchange of pollinators between forest and farmland (e.g. integration of Euglossini-attractant plant species, pollinator loans)
- Development of a direct and transparent supply chain with a central role for the cooperative Osacoop in capacity building and farmer organization.

The agroforestry designs (Appendix VI) take into account the spatial competition between vanilla tutor trees and other crops (e.g. cacao). Furthermore, they are flexible and user-friendly, and can be implemented on (a) fallow or degraded lands, or (b) transformed agricultural lands (e.g. oil palm and cacao plantations). Based on the included crops, an economic modelling has been carried out, yet, the designs need further evaluation in terms of both practical feasibility and sustainability. This includes the calculation of production, costs, and revenues on the one hand, and biodiversity indices on the other hand.

7.2 CONCLUSIONS AND FUTURE RESEARCH PATHS

7.2.1. Main conclusions

We now provide an overview of the main take-home messages (Fig. 7.6), followed by a brief discussion of study limitations and suggestions for further research.

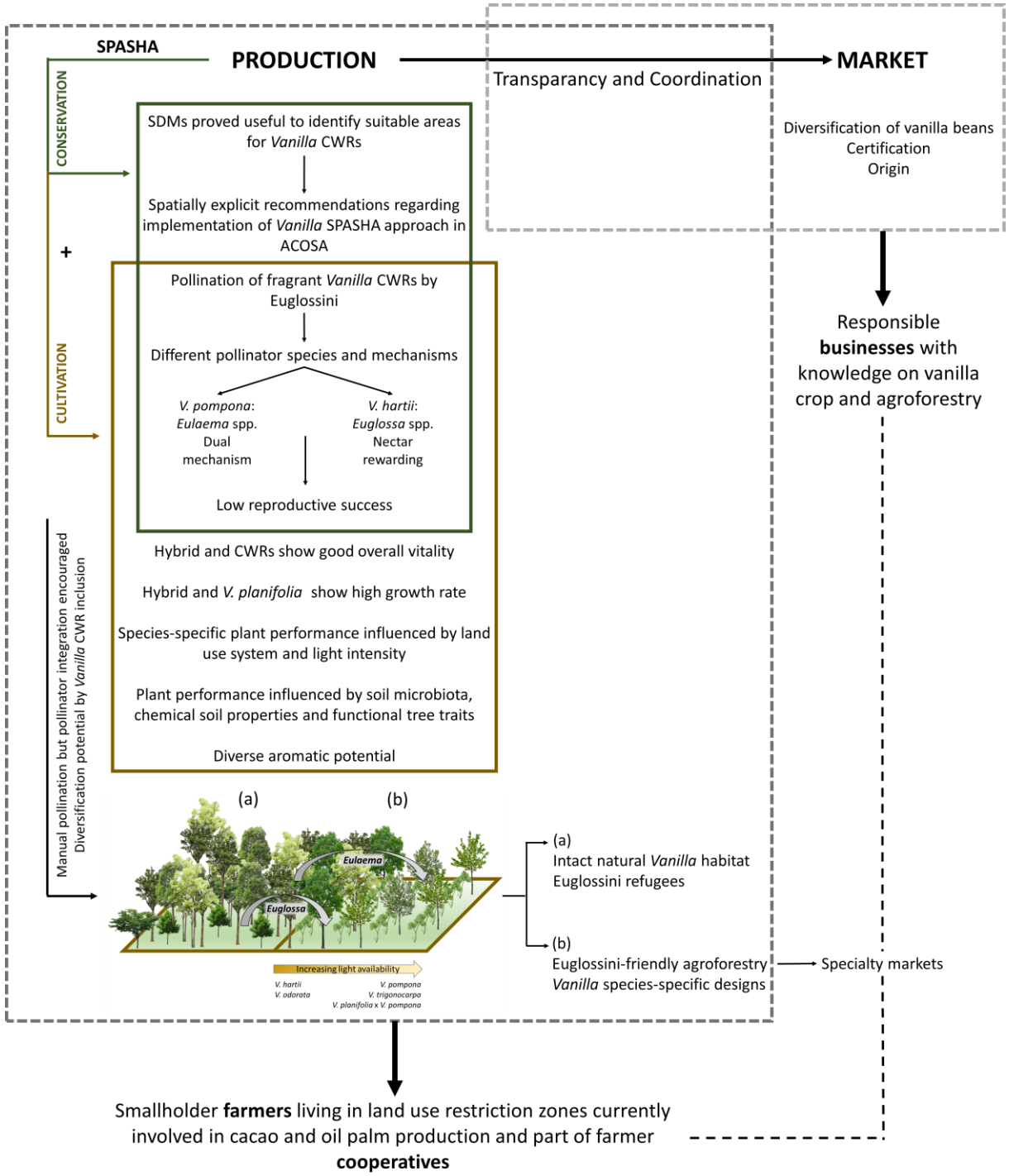

***Fig. 7.6**. Schematic overview of the main conclusions per work package.*

7.2.2. Study limiations and paths for further research

Species distribution models

One of the main limitations that we experienced when modelling the distribution of *Vanilla* CWRs growing in Costa Rica, was the restricted access to confidential species occurrence data. As such, we see great value in the formation of a **global *Vanilla* research network** that aims at enhancing the exchange of *Vanilla* species occurrence data through a **confidential data platform**. The latter may greatly facilitate the development of SDMs for a larger set of species.

Within the context and timeframe of this PhD thesis, we developed models using near current climate conditions (WorldClim 2.0), confining our knowledge on potential range shifts under the predicted climate change scenarios (CMIP5, Karger et al., 2017). We also assumed that, besides the integrated climate, soil, and topographic variables, the presence of pollinators, seed dispersers, soil microbiota, and mycorrhiza may further explain the observed *Vanilla* distribution patterns. This can be explained by the fact that (a) pollen and seed dispersers play an important role in a species' genetic diversity and thus adaptation capacity, and (b) both soil microbiota and mycorrhizal fungi are essential for vanilla seed germination and plant growth. We did not include these variables into the models due to the lack of data at that time. Since this PhD research (Chapters 3-4) and other recent studies (e.g. Pansarin, 2021; Pansarin & Ferreira, 2021; Pansarin & Suetsugu, 2022; Karremans et al., in prep.) have shed light on both pollination and seed dispersal mechanisms of some *Vanilla* species, **future SDM studies** could now integrate these **fundamental ecological interactions**. As such, we can further expand our knowledge regarding *Vanilla* distribution patterns and define integral conservation actions. Moreover, potential range shifts of *Vanilla* species and these biotic vectors under **predicted climate change scenarios** will provide insights into possible mismatches that may lead to increased extinction risks, especially for species with an already limited distribution. The species distributions modelled under future climate conditions may also be used to recommend optimal cultivation areas. Additionally, differences in suitability may be associated with **species-specific functional plant traits**, which were not included in our models due to the lack of available trait data. Supplementary studies could measure certain traits of interest (e.g. leaf dry matter content, leaf thickness and toughness, specific leaf area) that can subsequently be included into models to further enhance our understanding of interspecific variability in habitat suitability.

When applying SDMs, one will always have to deal with certain levels of model and data uncertainty. The extent of the modelling range is of particular importance, and has been a main point of discussion. We decided to collect occurrence data across the native growing range of

fragrant *Vanilla* species (i.e. Neotropics) to (a) include the full environmental variation under which a species can occur, and (b) to account for the scarcity of available georeferenced data on most *Vanilla* species. Subsequently, we modelled the species at the extent of Costa Rica (selecting at least half of the presence points inside Costa Rica), and cropped the obtained distribution maps to our study region ACOSA. As such, the spatial uncertainties may be larger than our considered study region. To overcome this problem, a zonal instead of a grid cell approach may be preferred, using both the results from the models and detailed forest inventories. For example, *V. hartii* is mostly restricted to the old-growth forests and forest ridges, whereas *V. pompona* has a high adaptation capacity and may occur in various types of habitats. If land use maps indicate the presence of an old-growth forest in a certain area of ACOSA, but the model shows grid cells with both high and low suitability, zonation may facilitate the interpretation and practical implementation of, in our case, the case-specific *Vanilla* SPASHA approach. Hence, a combination of SDM with detailed field observations and landscape patterns would be ideal to provide spatially explicit recommendations for the application of certain approaches at smaller scales.

Vanilla pollination studies

As expected, the the vanilla pollination studies were time-consuming. We originally aimed at identifying the pollinators of the four modelled *Vanilla* species (*V. hartii*, *V. odorata*, *V. pompona*, and *V. trigonocarpa*), yet, were only able to obtain insights into the pollination mechanisms of two of these species. Based on our results and an in-depth literature review, we assume that the allogamous species belonging to *Vanilla* sect. *Xanata* are pollinated by Euglossini. As such, a range of Euglossini species could be collected and placed together with flowering *Vanilla* species in **enclosed flying cages** to optimize and accelerate data collection.

Furthermore, the discussed insights into plant-pollinator interactions within the *Vanilla* genus need to be corroborated by research on other *Vanilla* species to reveal **specific evolutionary trajectories** within this genus (e.g. evolution of rewarding and rewardless mechanisms). In this context, we see great value in studies that continue to identify *Vanilla* pollination mechanisms by combining observational data on pollinator species, their behaviours, pollination success and flower morphology, with phytochemical analyses of nectar, floral volatile compounds, and other potential floral rewards. Next, examining possible **associations between vanilla bean size, flower to bean ratio (fruit set) and corresponding pollination mechanism** may further disclose potential evolutionary trends. For example, it is possible that species producing large and/or thick fruits (e.g. *V. pompona*) can only bear a limited number of fruits due to the high energy

demand related to fruit development. Hence, these species display dual/food-deceptive mechanisms known to result in a lower fruit set. On the contrary, species producing small and thin fruits (e.g. *V. hartii*) evolved a nectar-rewarding mechanism that leads to higher pollination efficiency. The above hypothesis is based on the assumption that large fruits contain more seeds, so the actual reproductive success of species producing few but large fruits could be similar to that of species with more but small fruits. Moreover, further assessment of ***Vanilla* and pollinator habitat requirements** is necessary to better understand potential ecological adaptations that may have driven the evolution of different pollination mechanisms within the *Vanilla* genus, thereby also supporting the development of pollinator-friendly vanilla cultivation systems.

Finally, we see great value in studies that compare the **quality of naturally with manually pollinated vanilla beans**. Initial results from a pilot study in Mexico (personal communication with Dr. Araceli Pérez Silva) indicate higher aromatic potential of naturally pollinated beans, but in-depth investigation on a variety of species is needed to provide more insights herein.

Experimental field sites for CWR cultivation potential

One of the main limitations of our field experiment was the lack of production data, as *Vanilla* species generally start to produce three to four years post-planting. Three CWRs showed early-flowering phenotypes, an interesting trait that requires further examination. Yet, no sufficient data could be collected to draw conclusions regarding production and associated revenues. If we had implemented the field sites one year earlier (2018 instead of 2019), we may have had the chance to collect more data. Unfortunately, this was impossible due to preparations that had to be made regarding field site selection, study design, and research permits.

Our study focused on four CWRs, yet, there are at least 37 fragrant *Vanilla* species present in the Neotropics. Continued research involving **other species** is highly required to fully capture the potential contribution of *Vanilla* CWRs to crop improvement and breeding. Furthermore, **controlled experiments** that evaluate specific biotic (e.g. pathogens) and abiotic (e.g. climate change-induced changes in temperature, precipitation) stress resistance are highly recommended, alongside the **characterization of the aromatic potential and production** of these fragrant CWRs. From here, further recommendations can be given on the integration of a mix of resistant and highly productive species within mixed farming systems. Furthermore, **hybridization** between promising *Vanilla* CWRs could lead to the development of varieties that are stress-resistant, highly productive and hold satisfying organoleptic features, with the hybrid used in this study as a great example.

The lack of time and funding restricted us from identifying the mycorrhizal fungi associated with the *Vanilla* species under study. Alike other orchids, vanilla has a strong relationship with mycorrhizal fungi for, amongst others, seed germination and plant growth. Additional research should include data on the **presence of both soil microbiota and mycorrhiza,** with a comparison among *Vanilla* species and land use systems.

Discrete choice experiment

Discrete choice experiments (DCE) are the preferred choice if one aims at exploring preferences towards a hypothetical situation, i.e. in the absence of revealed preference data. DCEs facilitate a systematic data anlysis using a sequence of statistical models (conditional logit, mixed logit, latent class), and allow one to gather data of a larger number of respondents. Yet, it often restricts the collection of additional data (e.g. socio-economic aspects) crucial to fully interpret the obtained results. We intented to overcome this limitation by adding a short quantitative survey at the beginning of the DCE. However, a **combined quantitative and qualitative survey** (i.e. with closed and open questions) may have been a better option to gain more detailed insights into the socio-economic situation at respondent level.

Since vanilla is a rather new crop in our study region, stated preference surveys may lead to a so-called hypothetical bias (see Chapter 6 – Section 6.5). We limited this bias by (a) including a short introduction video at the beginning of the survey, and (b) explaining that there are no right or wrong answers. Two additional methods could be used in the future to further **limit the hypotehtical bias**. First, respondents can be asked how sure they are about their choices, filtering out the rather questionable ones. Second, respondents can be asked to choose their preferred option from a choice set of two hypothetical alternatives. Afterwards, they are asked to compare their choice with the opt-out option. The effectiveness of these methods, however, is still under debate.

To make the choice situations as realistic as possible, we added certain contraints in the experimental design. This, however, may have caused the observation of non-significant revenue effects. Reducing the number of constraints or making them less strict, may result in a larger number of revenue combinations, enabling us to retrieve information on farmers' preferences regarding revenues, and to determine the **willingness to pay**. Furthermore, although predictions were made about the signs of the priors used in our experimental designs, they did not correspond with the signs of the parameter estimates of our results. A **pilot study** prior to the survey may allow a better specification of the Bayesian priors, leading to a higher design efficiency.

The SPASHA approach

Based on the observed plant performance (survival, vitality, growth), the presence of early flowering phenotypes, and the identified vanilla bean aromatic compounds, we see potential for integration of *Vanilla* CWRs within a production context. According to the proposed *Vanilla* SPASHA approach, we recommend to transform fallow or existing agricultural lands (e.g. cacao plantations) to vanilla agroforestry systems, taking into account the observed (species-specific) effects of certain environmental variables on vanilla plant performance. Within the timeframe of this PhD thesis, however, we could not collect data on production, costs and revenues at both vanilla species and system (including other crops such as cacao) level. This information is of the essence if we want to develop practical guidelines for the implementation of the *Vanilla* SPASHA approach, and for its promotion as an optimal and biodiversity-friendly production strategy for the vanilla crop within its centre of origin. As such, an **evaluation of the feasibility** of the SPASHA approach in terms of production, costs and revenues as well as an **evaluation on its functionality within the context of climate change mitigation and biodiversity conservation** is highly required. The latter, for example, refers to the functioning of the proposed vanilla cultivation systems as biological corridors between the protected areas, and could be measured by examining a range of species diversity indices. A system-level comparison between intensively managed vanilla monocultures and mixed farming designs including a variety of *Vanilla* species and other crops will give further insights about the potential economic and environmental benefits of this concept of joint land sparing/sharing as a possible strategy for sustainable vanilla production, orchestrating humand land use with biodiversity conservation.

We did not explore the possibilities of *ex situ* conservation actions for the *Vanilla* CWRs studied in this PhD. As such, an evaluation of *ex situ* strategies is highly recommended. This could start with an ideally regional **genotyping-by-sequencing program** of both wild *Vanilla* populations and already existing *ex situ* collections (e.g. gene banks, botanical gardens). A combination of gene sequencing (e.g. using SNP markers) and controlled experiments with *in vitro* material from these *ex situ* collections could vastly contribute to disentangle the genetic diversity available among the different *Vanilla* species and populations, thereby providing a **baseline for further evaluation of CWR use in crop improvement and breeding programs**.

Finally, it is important to validate the possible **monetary compensation** regarding the *Vanilla* SPASHA approach. For example, farmers implementing the proposed land use strategy could receive Payments for Ecosystem Services (PES) from the Costa Rican Fondo Nacional de Financiamiento Forestal (FONAFIFO), as they are protecting forests present on their farms and

(potentially) holding wild *Vanilla* populations. This mechanism has been proposed by Tyack et al. (2020) to encourage *in situ* CWR conservation actions. However, to promote the cultivation of new vanilla varieties as an attractive cash crop within this approach, an **evaluation of *Vanilla* CWR market value** is required. Taking into account the current limitations on commercial *Vanilla* CWR use, only the pods of the hybrid and *V. planifolia* can be harvested for commercial purposes, and continued research is needed to complement our initial observations. In this context, the **legalization of *Vanilla* CWR use** and the **transformation of current market regulations** will be crucial to enable the integration of these new varieties within a commercial production context. Support from and collaboration among institutions such as Crop Trust, Alliance Bioversity-CIAT, CIRAD and other international, national, and local organizations will be of great value to facilitate abovementioned processes.



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Photo: *Erythrina* trees with recently planted cuttings of the hybrid (*V. planifolia* x *V. pompona*) *x V. planifolia*, as part of the model farm established at the farmer cooperative Osacoop in the Osa Peninsula. © Charlotte Watteyn

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Appendices

Photo: The hybrid (*V. planifolia* x *V. pompona*) *x V. planifolia*, growing around an oil palm, implemented by a farmer living in the Osa Peninsula. The method may have great potential for the diversification of oil palm plantations.

APPENDIX I

Supplementary information to Chapter 2 – Vanilla distribution modelling for the conservation and sustainable cultivation a joint land sparing / sharing context

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Table A1.1. The mean AUC values for the individual model algorithms, and their mean weights, included in the ensemble modelling of *Vanilla odorata*.

Algorithm	Mean AUC	Mean weights
ENSEMBLE	0.9096076	0.00000000
MAXENT	0.8591379	0.11822675
SVM	0.8340014	0.12702263
GLMNET	0.8123647	0.10836650
GAMSTEP	0.8069341	0.06724613
DOMAIN	0.7985309	0.09927350
FDA	0.7971939	0.08269150
MGCV	0.7791055	0.07776263
EARTH	0.7634215	0.06720950
GBM	0.7563492	0.06333300
RF	0.7551764	0.06236437
GLMSTEP	0.7499729	0.07654000
GBMSTEP	0.7479167	0.04996387

Table A1.2. The mean AUC values for the individual model algorithms, and their mean weights, included in the ensemble modelling of *Vanilla pompona*.

Algorithm	Mean AUC	Mean weights
ENSEMBLE	0.8961042	0.00000000
SVM	0.8489812	0.13043150
GBMSTEP	0.8264366	0.08692762
MAXENT	0.8248881	0.08718237
GBM	0.8184199	0.08903437
RF	0.8182769	0.12689025
GLMNET	0.7916438	0.11025113
GAMSTEP	0.7740264	0.05726987
GLMSTEP	0.7702432	0.05804400
FDA	0.7404791	0.04518838
MGCV	0.7042061	0.04249125
EARTH	0.7030210	0.08278362
DOMAIN	0.6983300	0.08350525

Table A1.3. The mean AUC values for the individual model algorithms, and their mean weights, included in the ensemble modelling of *Vanilla hartii.*

Algorithm	Mean AUC	Mean weights
ENSEMBLE	0.9497863	0.00000000
SVM	0.9160562	0.13373050
MAXENT	0.8900663	0.12887525
GLMSTEP	0.8817700	0.10209225
GLMNET	0.8645343	0.12567700
MGCV	0.8553114	0.09883800
GBM	0.8548099	0.09633200
RF	0.8301173	0.06385750
GAMSTEP	0.7649573	0.09137325
DOMAIN	0.7584380	0.05570450
FDA	0.7386076	0.03409900
GBMSTEP	0.7281092	0.03338850
EARTH	0.6892007	0.03603100

Table A1.4. The mean AUC values for the individual model algorithms, and their mean weights, included in the ensemble modelling of *Vanilla trigonocarpa*.

Algorithm	Mean AUC	Mean Weight
ENSEMBLE	0.9342593	0.00000000
MAXENT	0.9111111	0.12056700
GBMSTEP	0.9062831	0.10238883
RF	0.9032407	0.11943650
DOMAIN	0.8720289	0.09732517
FDA	0.8652116	0.08155733
GBM	0.8592593	0.10273617
SVM	0.8577381	0.09724067
MGCV	0.7728887	0.03941583
GLMSTEP	0.7727564	0.05491733
EARTH	0.7686864	0.05801367
GAMSTEP	0.7625000	0.05604683
GLMNET	0.7584758	0.07035467

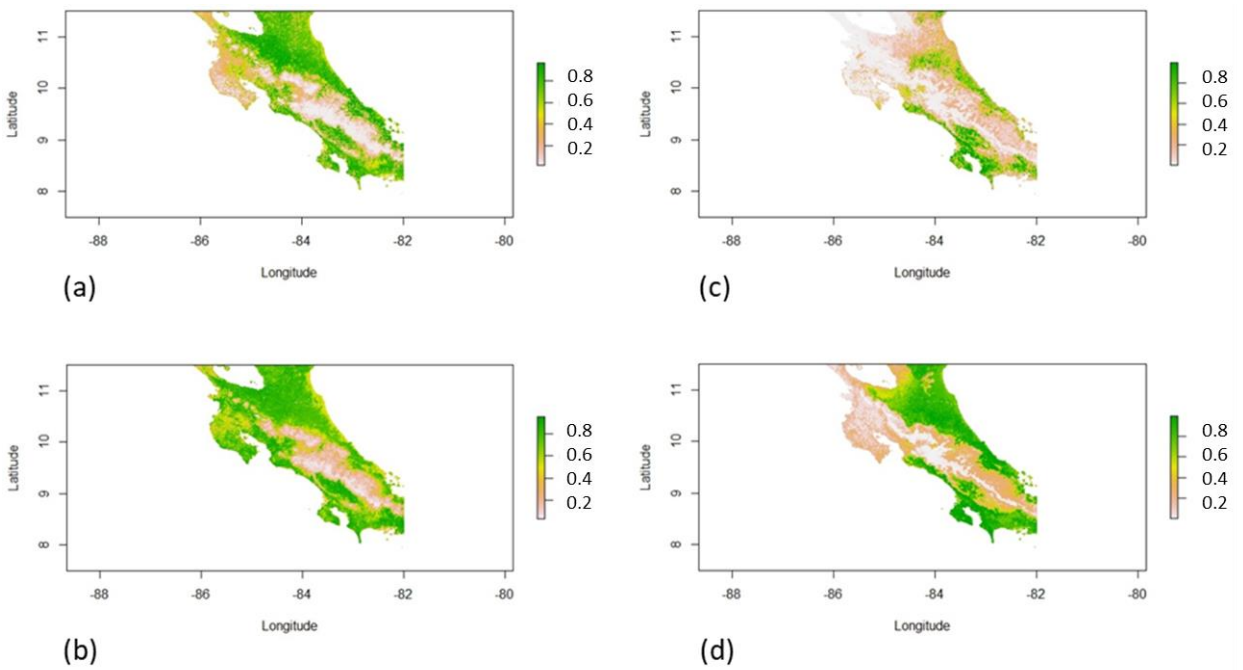


Figure A1.1. Suitability map for (a) *Vanilla odorata*, (b) *Vanilla pompona*, (c) *Vanilla hartii*, and (d) *Vanilla trigonocarpa* in Costa Rica, representing areas ranging from 0 (totally unsuitable) to 1 (fully suitable).

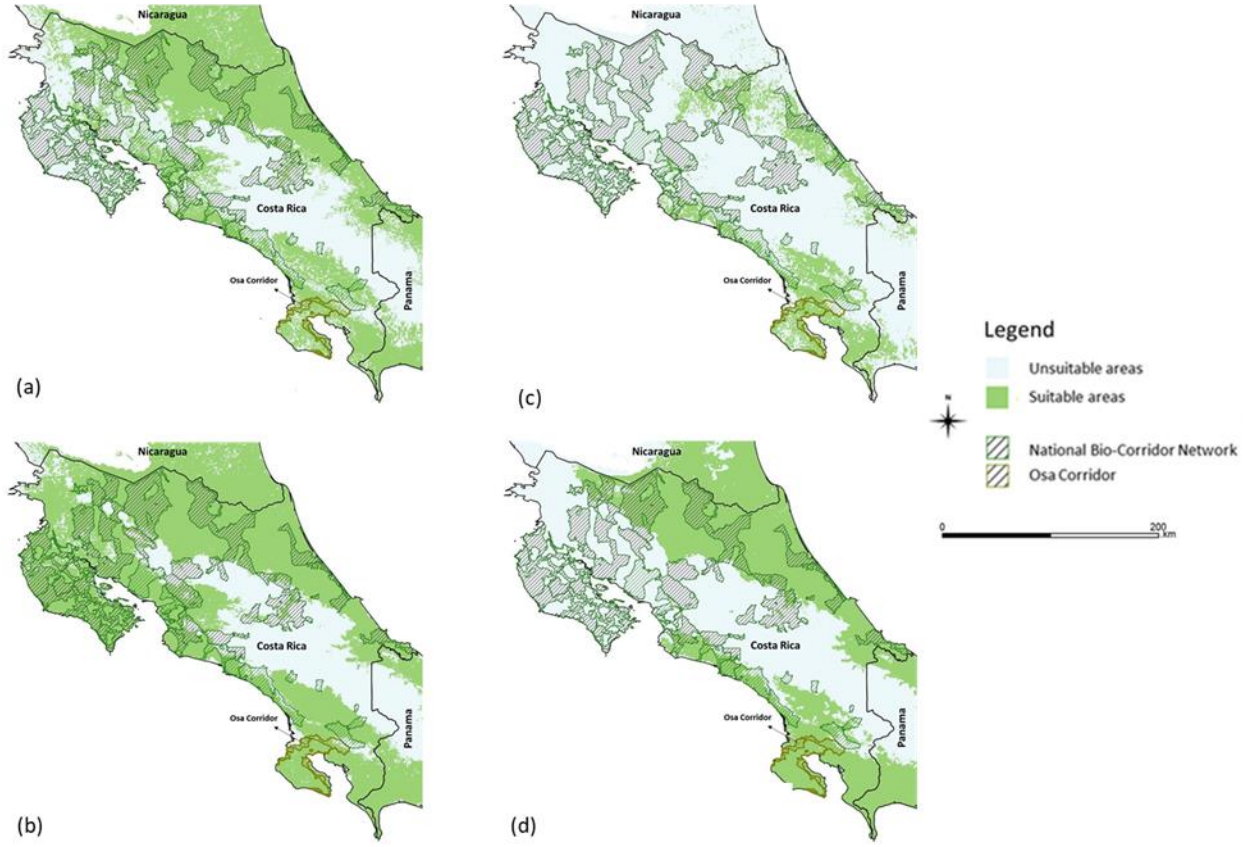


Figure A1.2. Map displaying the corridors that are expected to be suitable for (a) *Vanilla odorata*, (b) *Vanilla pompona*, (c) *Vanilla hartii* and (d) *Vanilla trigonocarpa,* in Costa Rica, with the Osa Corridor as focus area for this study. The light green areas are the areas suitable for the species in question to occur.

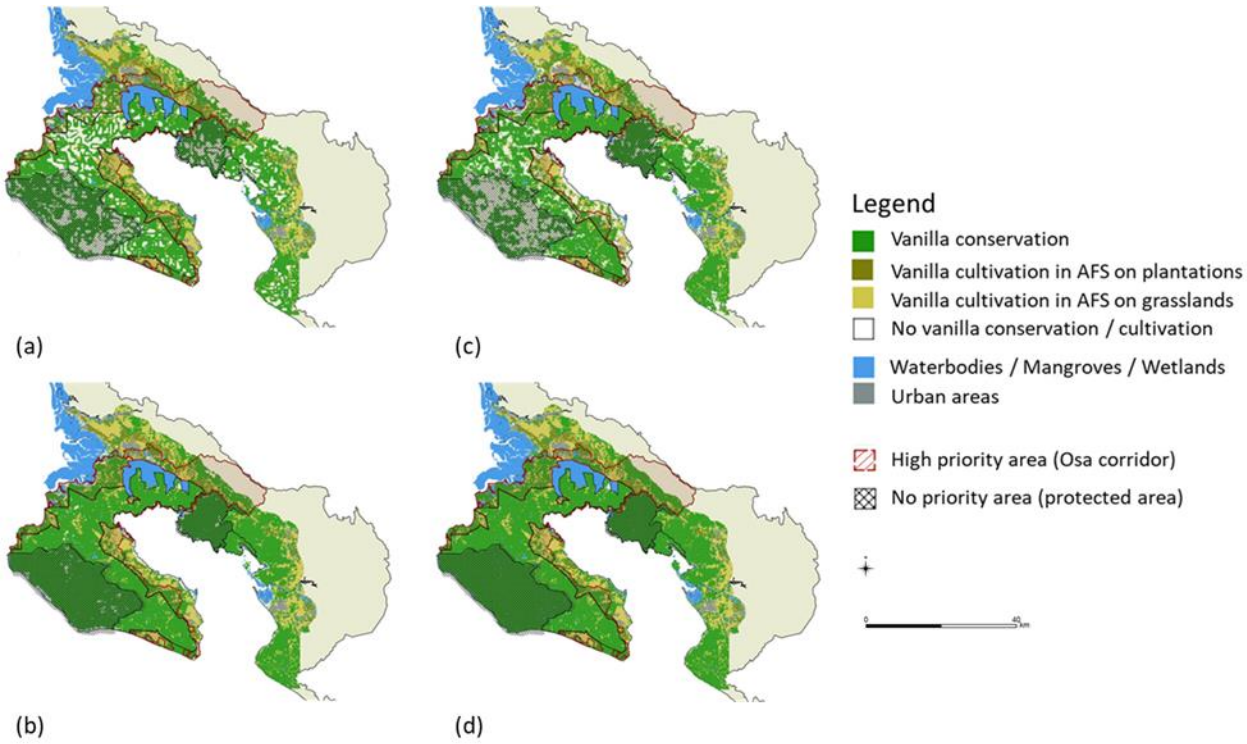


Figure A1.3. Map highlighting areas for conservation and sustainable cultivation of (a) *Vanilla odorata*, (b) *Vanilla pompona*, (c) *Vanilla hartii* and (d) *Vanilla trigonocarpa* within the ACOSA region. The green areas are forests suitable for vanilla to occur, giving these areas a vanilla conservation status, but the priority further depends on the location (in- or outside protected areas such as national parks). The brown and yellow areas are respectively plantations and grazing lands recommended for the implementation of sustainable agroforestry systems (AFS) with vanilla as a cash crop, especially in areas where natural vanilla populations (potentially can) occur in the nearby forests. The white areas are unsuitable areas for vanilla to occur, so with no recommendations regarding vanilla conservation or cultivation.

APPENDIX II

Supplementary information to Chapter 3 – Trick or Treat? Pollinator attraction in *Vanilla pompona* (Orchidaceae)

Table A2.1. Output of Bayesian multilevel behavioral comparison models to sequentially compare behavioral categories between floral visitor genera and time of the day. The intercept term is the bee genus *Euglossa* of the categorical variable bee genus. LOOIC = leave-one-out cross-validation, WAIC = Widely Applicable Information Criteria, *SE* = standard error, CI = credibility interval.

Behavioral comparison	LOOIC	WAIC	Parameter	Estimate	*SE*	95% CI
1. Landing versus approaching	74.6	64.9	Intercept	14.17	5.26	5.54, 25.97
			Eulaema	1.31	1.00	-0.58, 3.32
			Trigona	3.42	1.49	0.64, 6.46
			Time of the day	-1.16	0.49	-2.29, -0.39
2. Landing on labellum versus tepals	98.1	88.4	Intercept	-8.77	4.91	-21.60, -2.18
			Eulaema	6.49	3.86	1.87, 16.62
			Trigona	16.48	7.69	7.66, 37.14
			Time of the day	-0.01	0.21	-0.43, 0.38
3. Entering into labellum versus only landing	115.1	111.1	Intercept	-5.54	2.57	-11.61, -1.46
			Eulaema	3.33	1.85	0.58, 7.83
			Trigona	5.16	2.13	2.00, 10.36
			Time of the day	-0.01	0.13	-0.26, 0.23
4. Pollen removal versus only entering	39.8	35.7	Intercept	-4.70	4.57	-15.82, 2.96
			Eulaema	2.65	0.88	0.97, 4.43
			Trigona	-0.01	0.96	-1.91, 1.86
			Time of the day	0.12	0.47	-0.69, 1.25

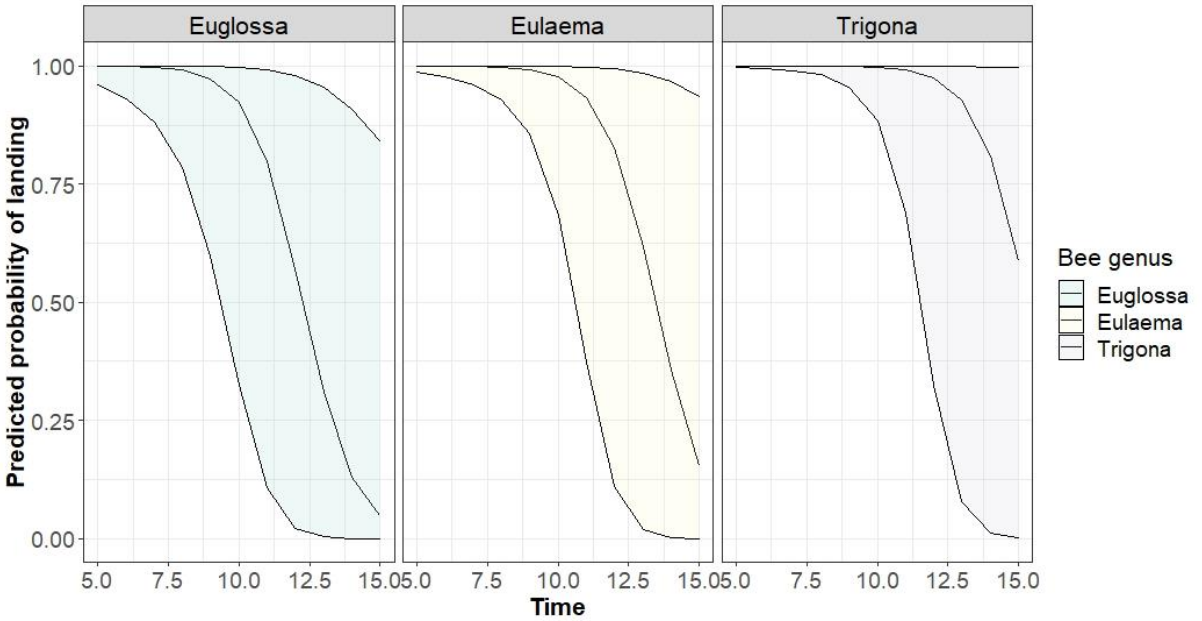


Fig. A2.1. Response curve of the behavior landing, shown over time and per bee genus; demonstrating the chance over time of the day that a bee individual belonging to a certain bee genus will land on a *Vanilla pompona* flower.

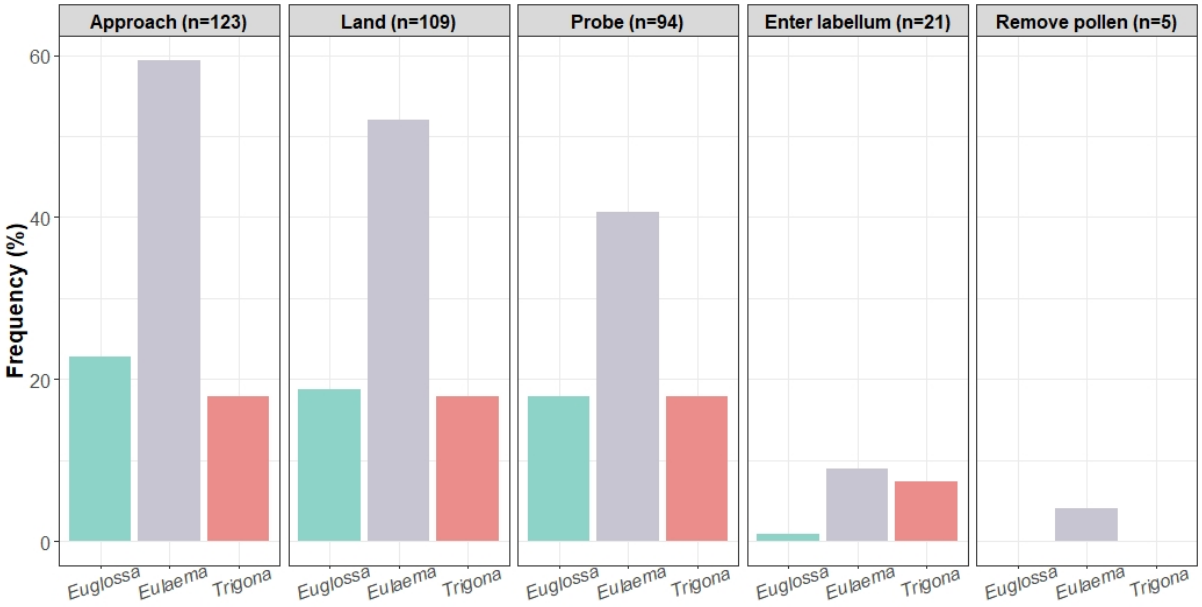


Figure A2.2. Percentage of floral visitors per bee genus that (1) approaches, (2) lands, (3) probes, (4) enters into the labellum, and (5) successfully removes pollen of *V. pompona* flowers.

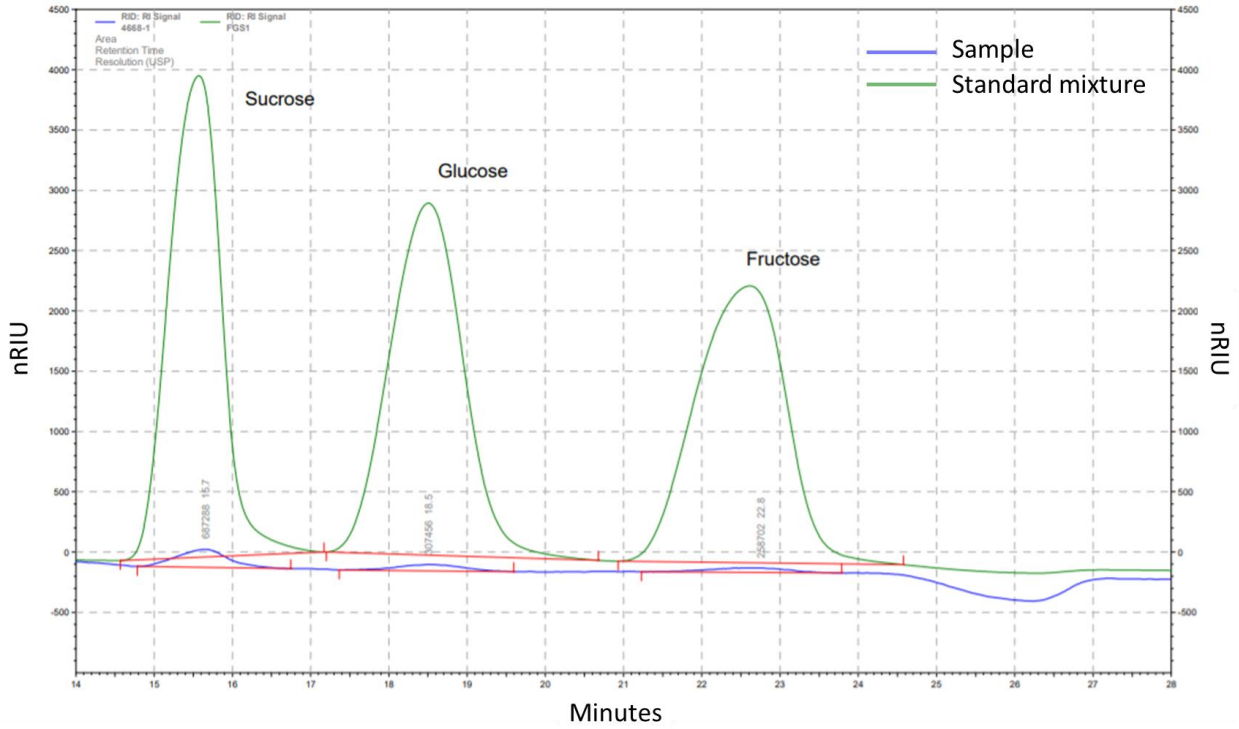


Fig. A2.3. HPLC-chromatogram of a *V. pompona* sample extracted from the labellum via three successive rinses (0.5 mL each) of de-ionized UHPLC gradient water. The green line represents a standard mixture of three sugars (sucrose, glucose, fructose). The blue line shows the sugar content of the sample. The limits of detection (LOD) was determined by the signal to noise (S/N) ratio method and estimated as the minimum concentration providing a S/N ratio of 3:1.

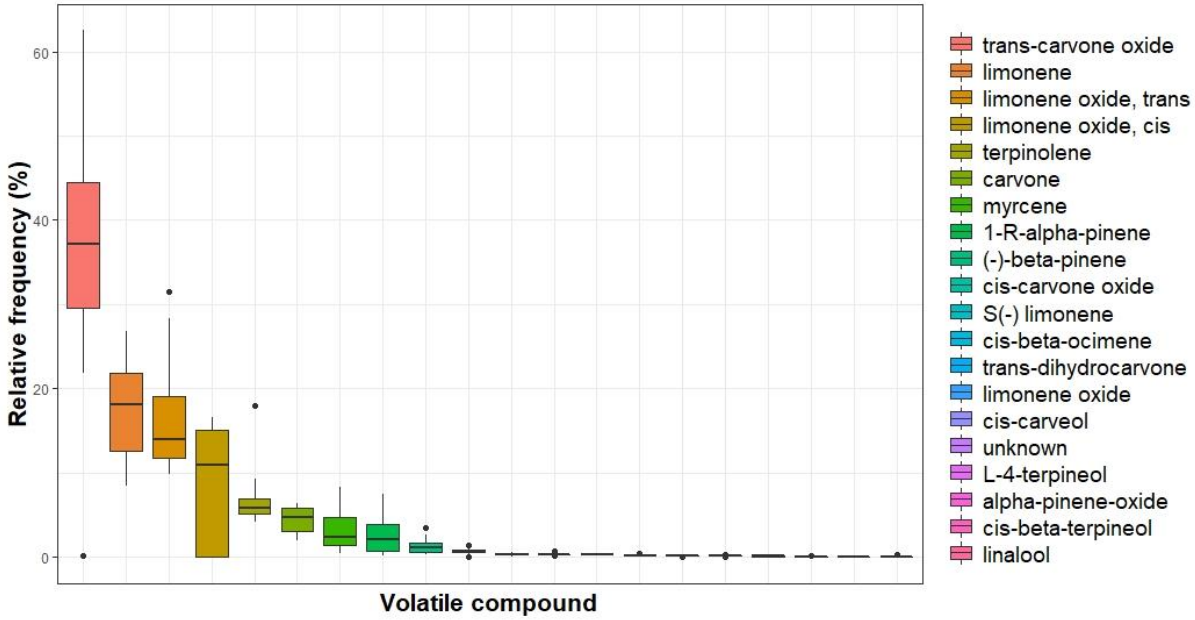


Fig. A2.4. Floral volatile compound composition of *V. pompona* flowers, calculated by averaging the relative frequency of each individual compound among the twelve samples.

APPENDIX III

Supplementary information to Chapter 4 - As sweet as *Vanilla hartii*: Evidence for nectar rewards in *Vanilla* (Orchidaceae) flowers



Fig. A3.1. Flying cage where individuals of different *Euglossa* species, caught at chemical baits, were placed together with *V. hartii* flowers. Their behaviour was observed during one hour, and individuals that entered the labellum and exited with pollen attached to their scutellum were identified at species level.

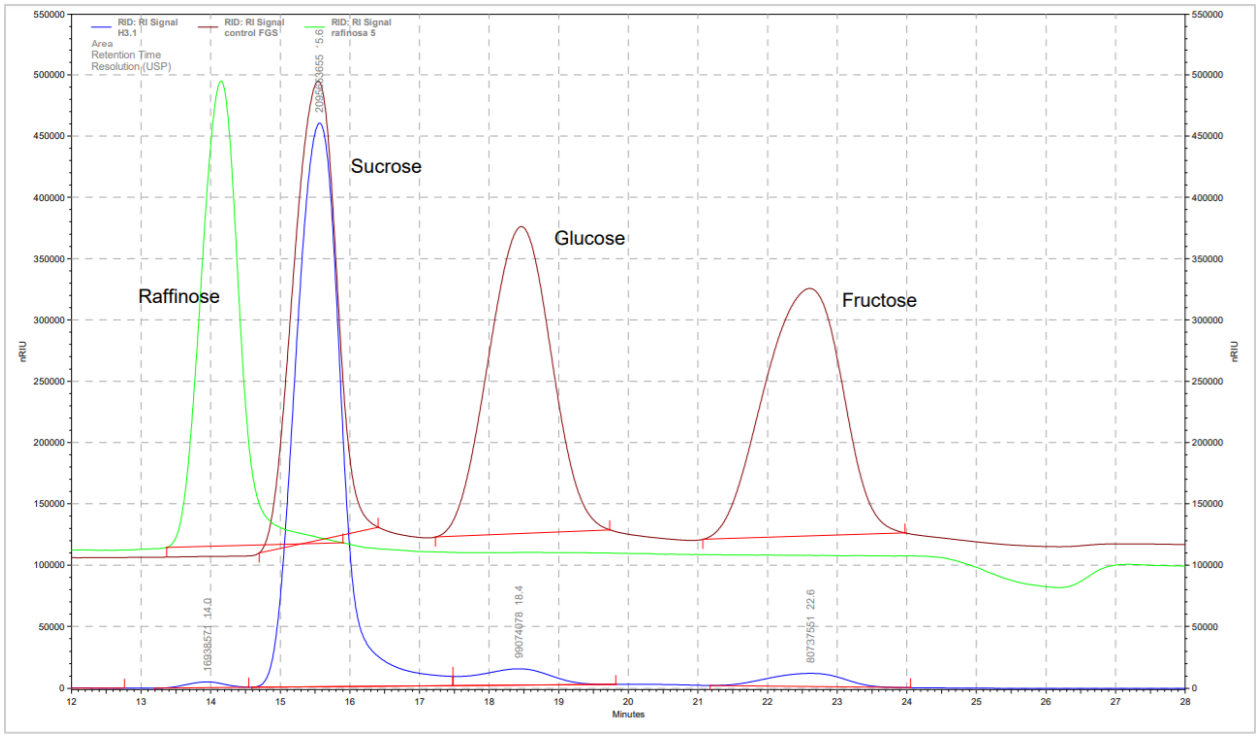


Fig. SA.2. Example of a chromatogram from the HPLC analysis of *V. hartii* floral nectar.

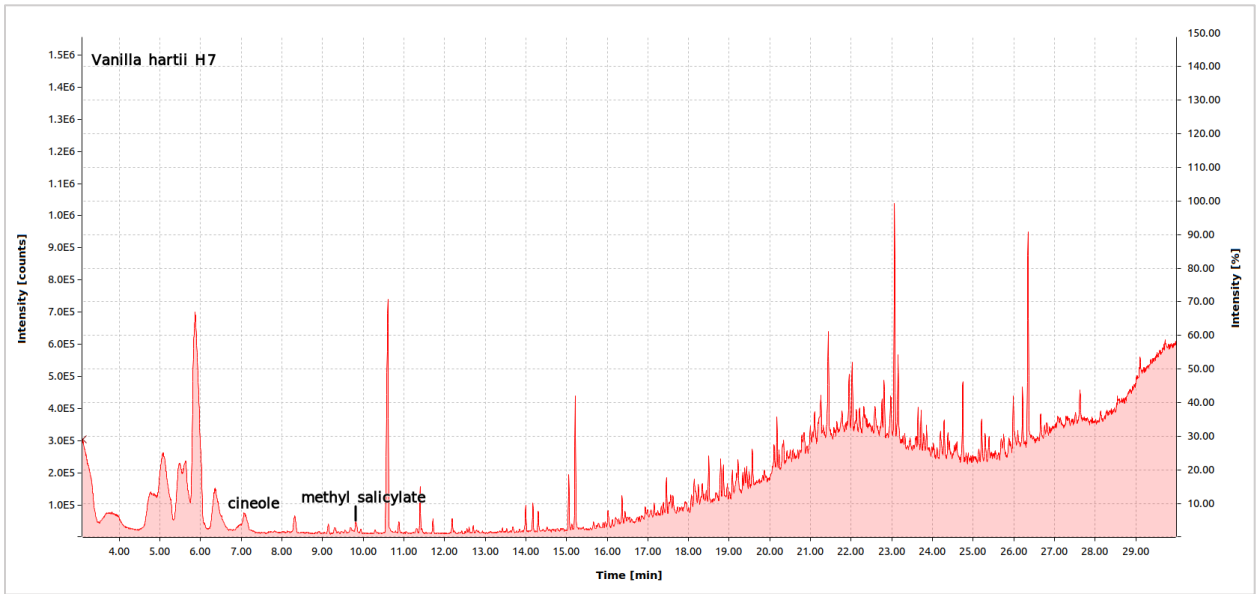


Fig. A3.3. Example of a chromatogram from the GC-MS analysis of *V. hartii* floral volatile compounds, indicating several peaks, yet only the small peaks corresponding to the compounds cineole and methyl-salicylate were identified as unique *V. hartii* floral volatile compounds, while the others represented noise (present in both sample and control).

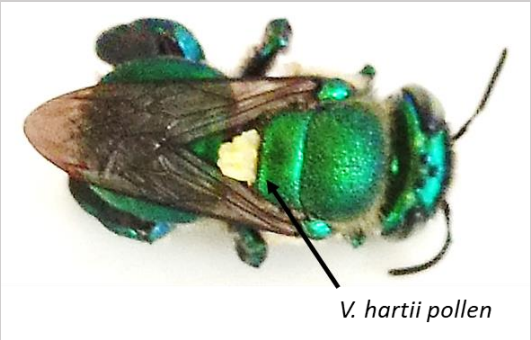


Fig. A3.4. *Euglossa tridentata*, identified after being observed entering the labellar tube of a *V. hartii* flower and effectively removing its pollen when placed in the flying cage. The pollen, indicated with the arrow, is typically deposited on the bee's scutellum. (voucher at JBL)

APPENDIX IV

Supplementary information to Chapter 5 – Initial insights on the cultivation potential of *Vanilla* crop wild relatives in two contrasting land use systems

Table A4.1. Overview of the measured plant performance response variables related to the survival, vitality and growth of the vanilla plants, as well as the derived variables.

Plant performance	Measured variable	Variable scores or indices
Survival		
	Plant survival	(1) Dead (2) Alive
Vitality		
Vegetative cutting	Condition vegetative cutting	(1) Necrosis (2) No necrosis
New shoot	Herbivore damage	(1) Strong (2) Medium (3) Low
	Pathogen presence	(1) Strong (2) Medium (3) Low
	Leaf discoloration	(1) Strong (2) Medium (3) Low
	Number of missing leaves	Count
	Number of missing shoot apices	Count
Growth		
	Soil roots	(1) Absent (2) Present
	Aerial roots	(1) Absent (2) Present
	New shoot	(1) Absent (2) Present
	Number of new shoots	Count
	Number of internodes per shoot	Count
	Internode length	cm
	Internode diameter	mm
	Leaf area	cm^2
Derived variables		
	Percentage of missing leaves	%
	Percentage of missing shoot apices	%
	Total shoot length	cm
	Total vine length	cm
	Mean internode length	cm
	Mean internode diameter	mm
	Mean leaf area	cm^2
	Total leaf area	cm^2

Table A4.2. Location (country, province, canton, district, village), pollination and harvest date of the sampled fruits per studied *Vanilla* species. All species belong to the genus *Vanilla* subg. *Xanata*, sect. *Xanata*. Longitude and latitude of the corresponding populations are not provided to protect these vulnerable populations, but can be shared upon request.

***Vanilla* species**	**Location**	**Flowering**	**Harvest**
Vanilla hartii	Costa Rica, Puntarenas Osa, Sierpe, Los Mogos	March 2020	November 2020
Hybrid	Costa Rica, Puntarenas Golfito, Puerto Jiménez, Gallardo	February 2020	October 2020
Vanilla helleri	Costa Rica, Puntarenas Golfito, Puerto Jiménez, Agujas	March 2020	November 2020
Vanilla odorata	Costa Rica, Puntarenas Golfito, Puerto Jiménez, Piro	July 2020	March 2021
Vanilla pompona	Costa Rica, Puntarenas Golfito, Puerto Jiménez, Piro	March 2020	November 2020
Vanilla trigonocarpa	Costa Rica, Puntarenas Golfito, Puerto Jiménez, Piro	June 2020	February 2021

Table A4.3. Results from the ANOVA-like differential abundance analysis of compositional data implemented in ALDEx2, demonstrating the bacterial phyla with significant differences in relative abundances among sites. kw.ep = expected P value of Kruskal-Wallis test. glm.ep = expected P value of glm test.

Phylum	**kw.ep**	**glm.ep**
Crenarchaeota	0.0000010562992835553	1.20321799238542e-12
Nitrospirota	0.00000327140095541853	4.85276595372591e-11
Cyanobacteria	0.000168892684161166	0.0000926972215764779
Dependentiae	0.000782206182709214	0.000936687372976761
Calditrichota	0.000913918425773585	0.000357608998016979
Bacteroidota	0.000975526294866611	0.0000360395123353767
Desulfobacterota	0.00145877317694087	0.0000695802663600615
Planctomycetota	0.0016152601396436	0.00000276694564428764
Verrucomicrobiota	0.00258192916953819	0.000000459906158930012
Methylomirabilota	0.00496770937022358	0.000726024245980427
Gemmatimonadota	0.00500913254624506	0.000373412278910155
Armatimonadota	0.00687087146614841	0.000890163151586021
Acidobacteriota	0.0112507551023068	0.000354359858033034
Myxococcota	0.0234925383556945	0.000243318124358967
Proteobacteria	0.0263951244762405	0.0208634826147982
Chloroflexi	0.027197584962376	0.00678056432183972
Latescibacterota	0.0352858165098403	0.0273635835589729
Firmicutes	0.0411968584589271	0.00772859342671875
Actinobacteriota	0.0463122664728378	0.0145795101803718



Fig. A4.1. Scoring of vanilla plants with relation to (A) herbivory, and (B) potential pathogens: insignificant (left), moderate (middle), high (right). Example of pictures of some of the commonly known pathogens used to compare in the field (C).

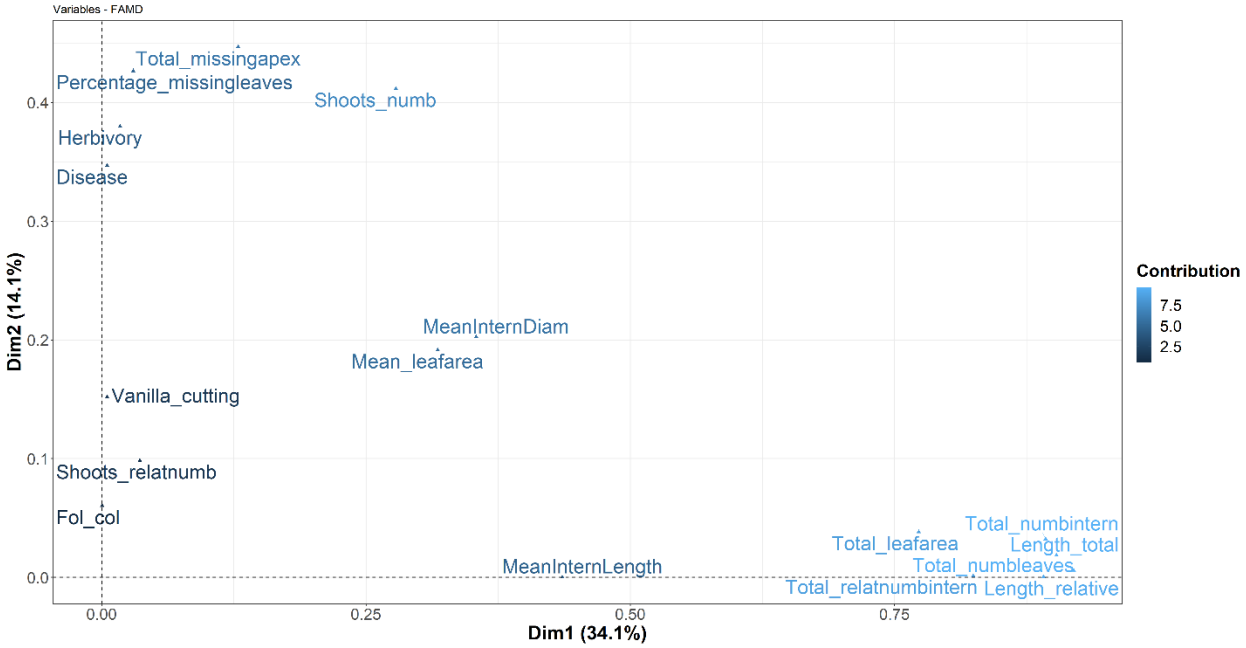


Fig. A4.2. Factor analysis of mixed data (FAMD) map displaying the categorical and continuous vanilla plant performance variables contributing to the first (34.1%) and second (14.1%) axis. The variables related to plant growth (e.g. number of internodes, internode length, etc.) correspond to the first axis, while the variables related to plant vitality (e.g. number of missing shoot apices, number of missing leaves, herbivory, etc.) correspond to the second axis.

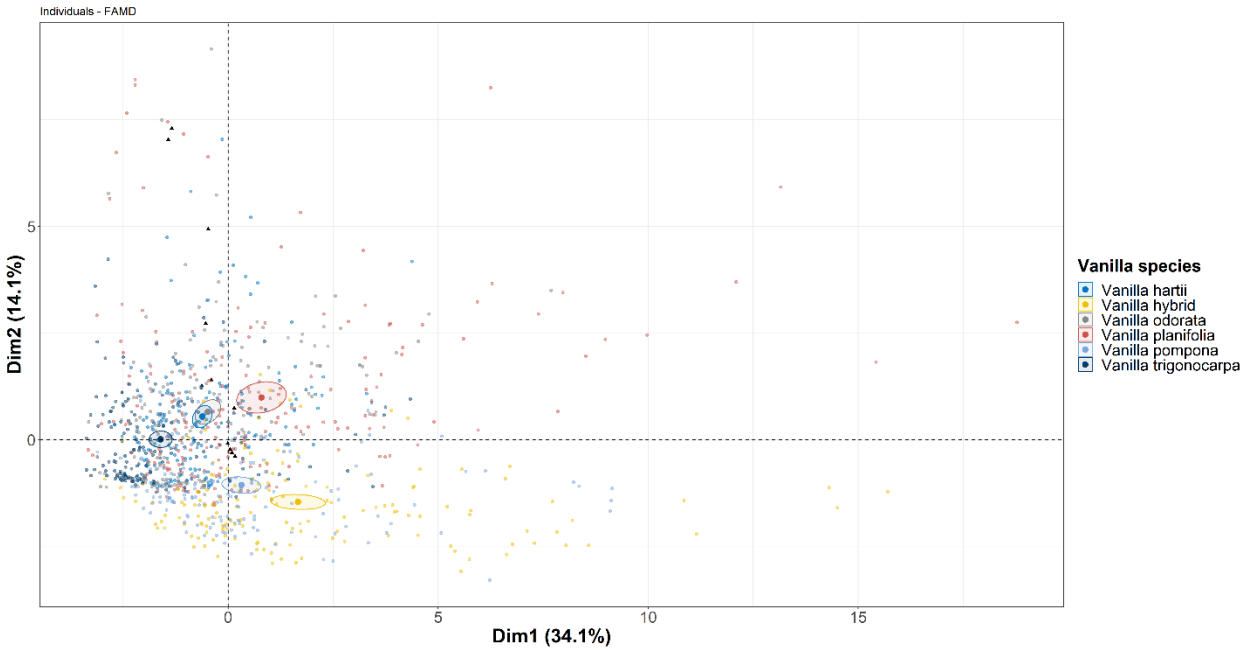


Fig. A4.3. Factor analysis of mixed data (FAMD) map showing the spread of the individual vanilla plants grouped per *Vanilla* species, whereby the plant performance variables related to plant growth and vitality correspond to the first and second axis, respectively. Note that increasing values of the first axis correspond with higher growth rates, while increasing values of the second axis correspond with lower plant vitality.

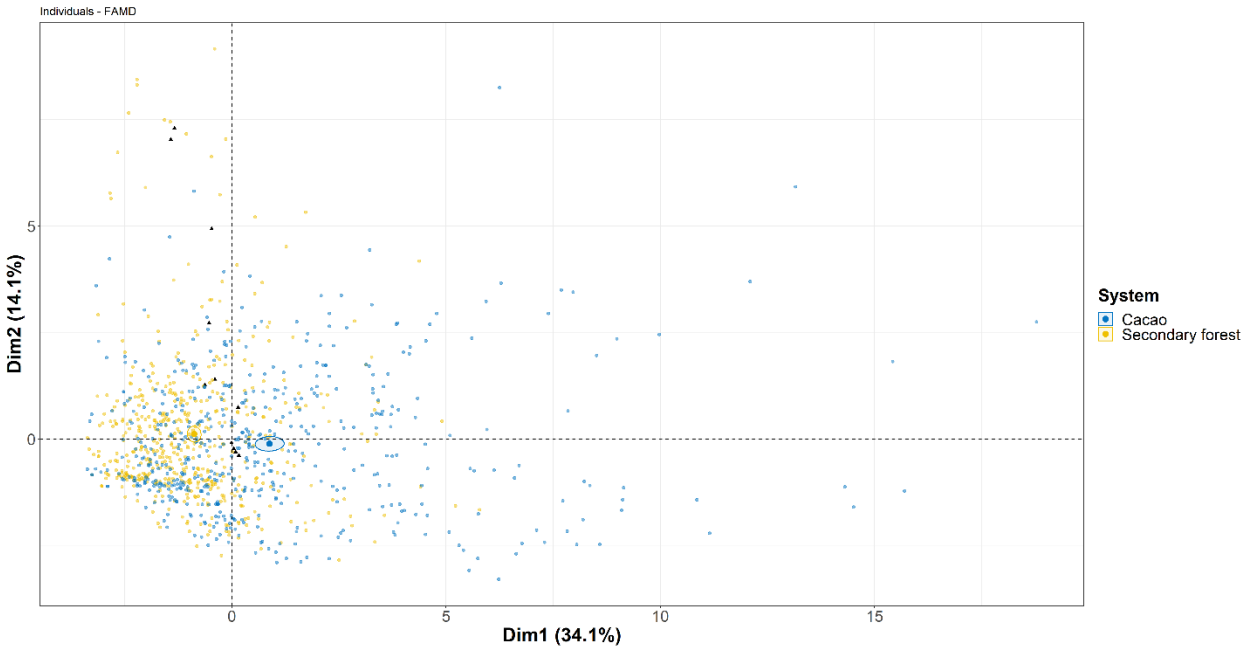


Fig. A4.4. Factor analysis of mixed data (FAMD) map showing the spread of the individual vanilla plants grouped per land use system, whereby the plant performance variables related to plant growth and vitality correspond to the first and second axis, respectively. Note that increasing values of the first axis correspond with higher growth rates, while increasing values of the second axis correspond with lower plant vitality.

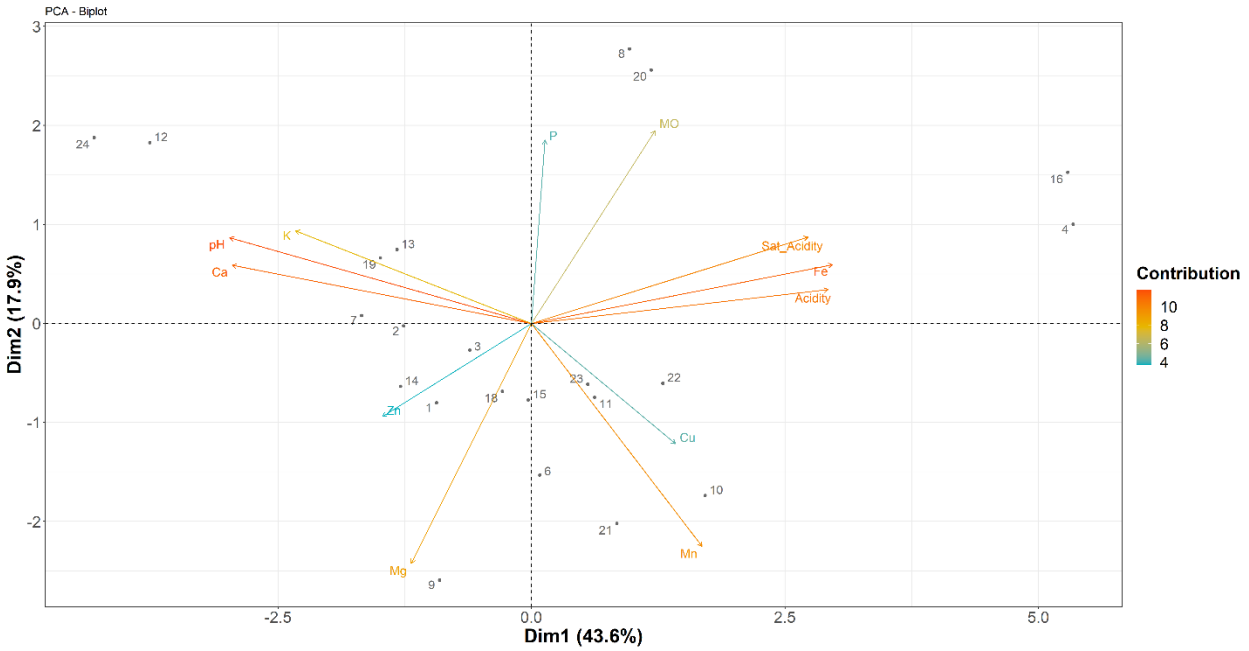


Fig. A4.5. Principal components analysis (PCA) biplot of soil chemical variables contributing to the first (43.6 %) and second (17.9 %) axis.

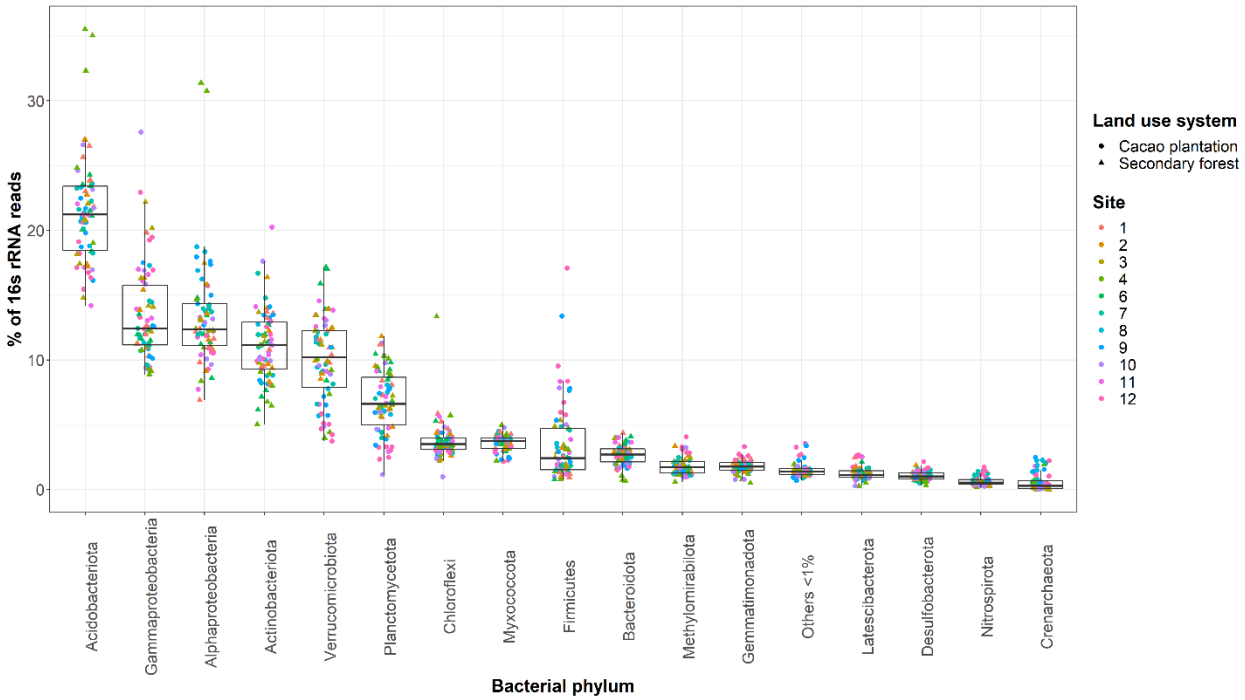


Fig. A4.6. Percentage of total 16S ribosomal RNA (rRNA) reads represented by the most abundant bacterial phyla, calculated per site. Sites 1 to 6 are located within secondary forests, and sites 7 to 12 in cacao plantations.

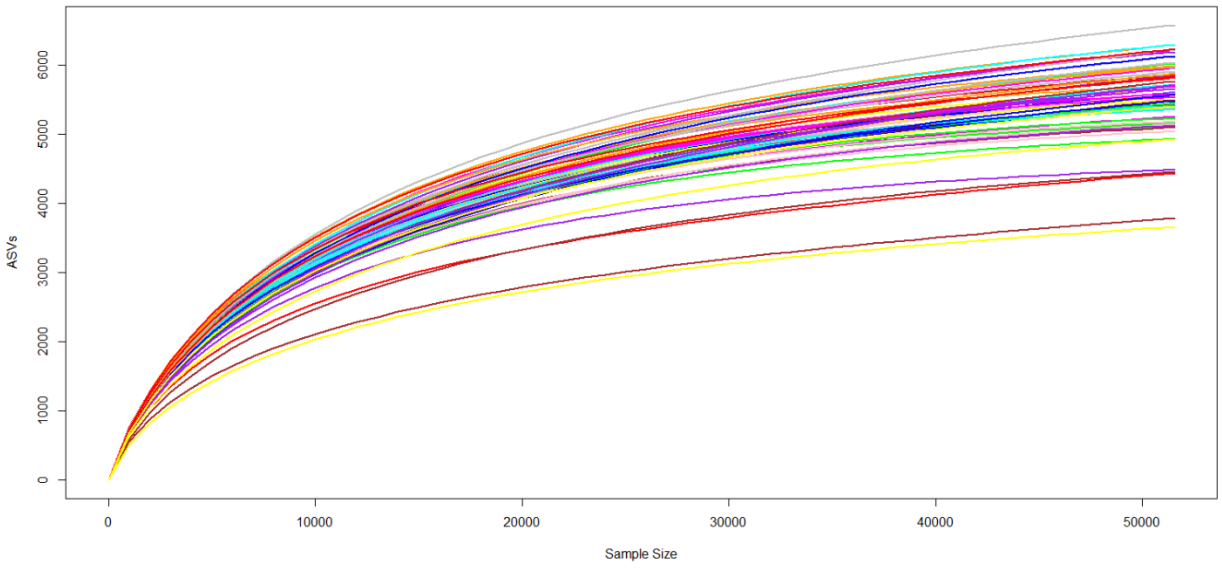


Fig. A4.7. Rarefaction curve to evaluate whether a specific sample has been sufficiently sequenced, demonstrating the number of species (ASVs) against the number of samples. It increases rapidly and slightly flattens, as the most common species are found first and the rarest species remain to be sampled.

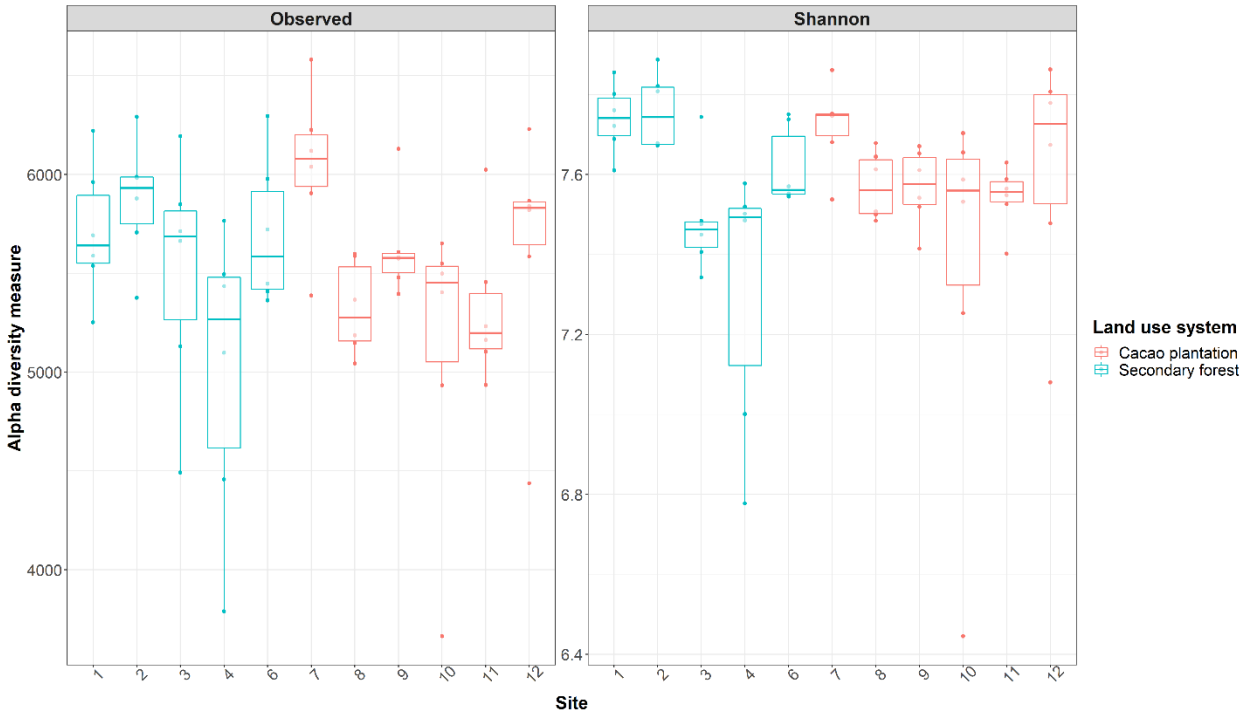


Fig. A4.8. Observed bacterial richness, i.e. number of unique ASVs (left) and Shannon-Wiener index of bacterial diversity (right) compared among site and land use system. Sites 1 to 6 are located within secondary forests, and sites 7 to 12 in cacao plantations.

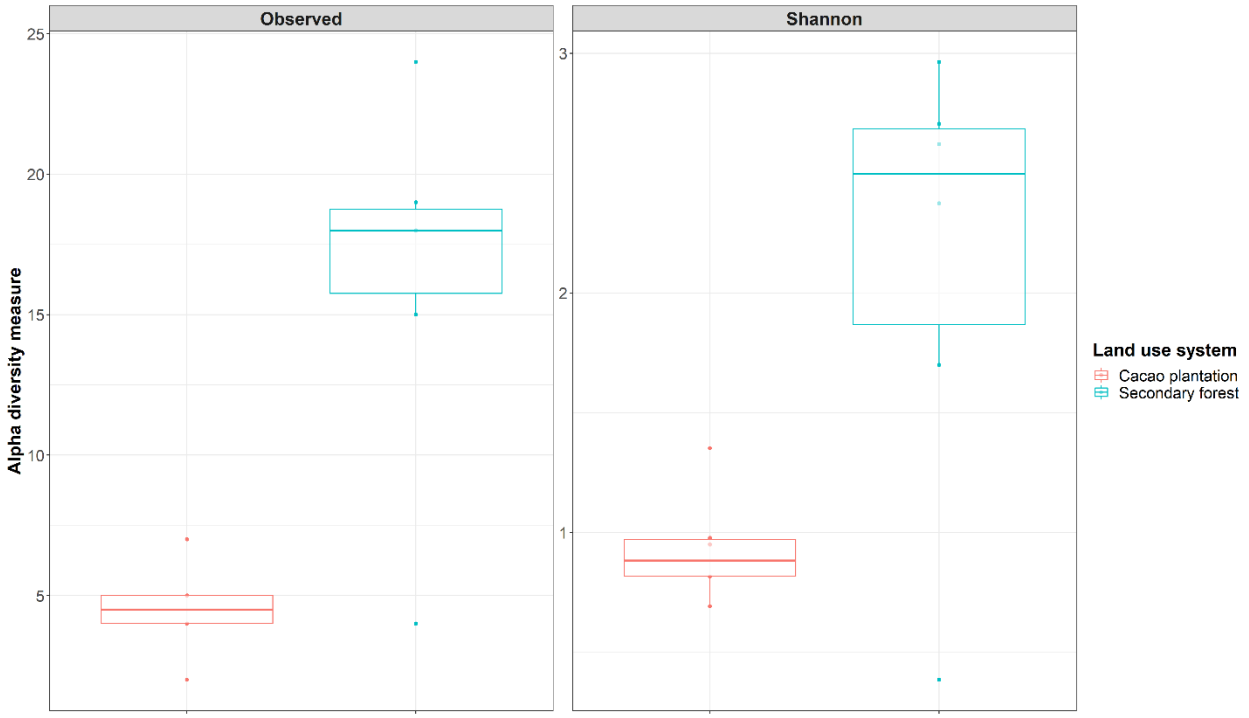


Fig. A4.9. Observed tree species richness (left) and Shannon-Wiener index of tree species diversity (right) compared among land use system.

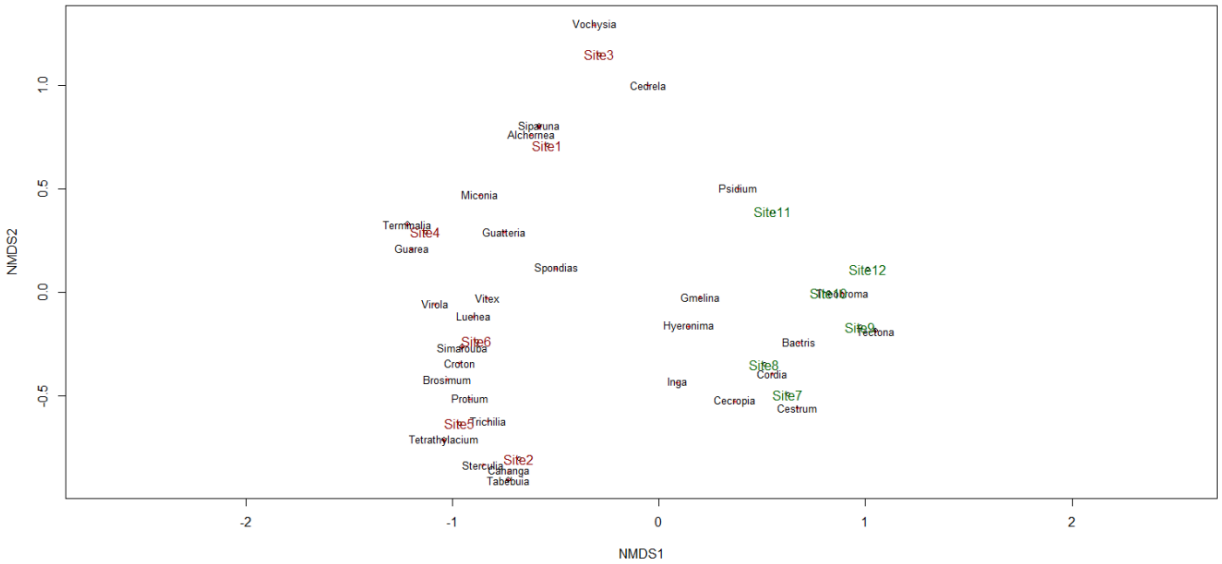


Fig. A4.10. NMDS plot demonstrating differences in tree species composition among land use systems and sites. Green = sites in secondary forest and red = site in cacao plantation.

APPENDIX V

Supplementary information to Chapter 6 – Exploring farmer preferences towards innovations in the vanilla supply chain

Table A5.1. Overview of the constraints. The first constraint rejects the combination of forest cultivation systems with highest revenues or intercropping agroforestry systems with lowest revenue. The second constraint avoids that cultivating CWRs generates the highest revenue and commercial species the lowest revenues. The third constraint rejects the combination of manual pollination with lowest revenues and natural pollination with highest revenues. The last constraint rejects the combination in which selling green vanilla beans would generate the highest revenues and selling cured vanilla beans would generate the lowest revenues.

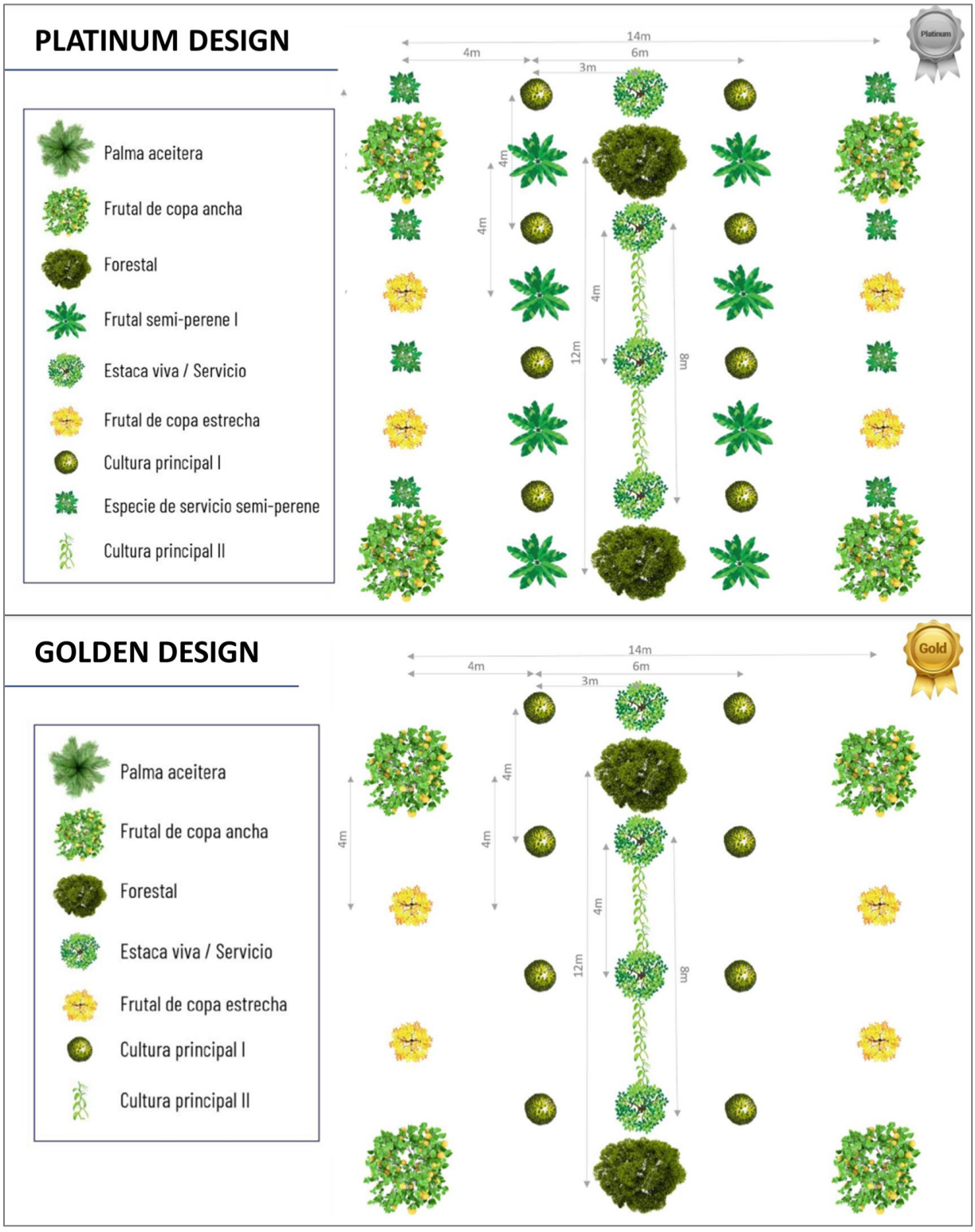
Constraint	Attribute	Level
1	Cultivation system | Revenue	Forest | $ 30,000
		Intercropping agroforestry | $ 1000
2	*Vanilla* species | Revenue	Vanilla crop wild relative | $ 30,000
		Commercial species | $ 1000
3	Pollination technique | Revenue	Natural pollination | $ 30,000
		Manual pollination | $ 1000
4	Post-harvest process | Revenue	Green vanilla beans | $ 30,000
		Cured vanilla beans | $ 1000

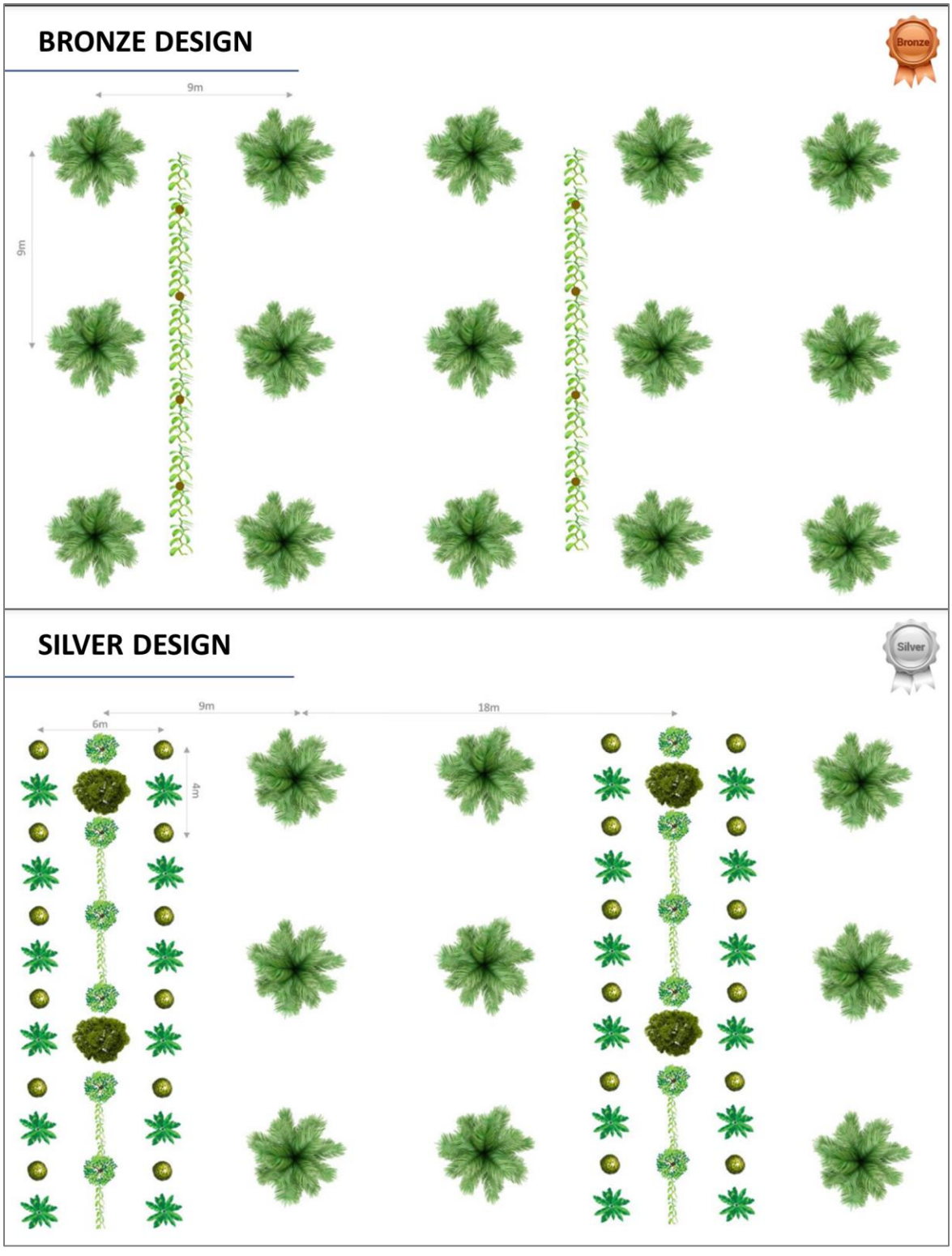
Table A5.2. Overview of respondents' agronomic and socio-economic characteristics.

	Option	Percentage (%)
Agronomic characteristic		
Area of land	< 25 ha	58.1
	> 25 ha	41.9
Presence of forest on land	Yes	73.1
	No	26.9
Presence of agroforestry on the land	Yes	38.2
	No	61.8
Income-earning activities on the land	Crop production	80.1
	Animal production	50.0
	Reforestation	12.4
	Without use	6.5
Cultivated crops	Cacao	9.1
	Palm	45.2
	Fruits and vegetables	43.6
	Other	37.6
Animal ownership	Cattle	42.5
	Other	24.2
Farm product purpose	Self-consumption	32.3
	Selling	67.7
Farm product destination	Local market	37.6
	National market	24.2
	International market	14.0
	No knowledge	8.6
Farmer cooperative membership	Yes	30.1
	No	69.9
Vanilla knowledge	Yes	62.9
	No	37.1
Vanilla commercialization	Yes	0.05
	No	99.5
Socio-economic characteristic		
Gender	Male	72.6
	Female	27.4
Age	< 50 years	62.4
	> 50 years	37.6
Education	None	25.3
	Primary school	59.7
	Secondary school	11.8
	Higher education	3.2
Monthly income	< 250,000 CRC	58.6
	250,000-500,000 CRC	28.0
	500,000-750,000 CRC	1.6
	> 750.000 CRC	2.7
	No information	9.1
Farmer location		
Location of farm in the Península de Osa	Outside land use restriction zone	31.2
	Inside land use restriction zone	
	- Biological corridor	45.7
	- Forest reserve	23.1

APPENDIX VI

Supplementary information to Chapter 7 – Agroforestry designs developed in collaboration with Pretaterra, Verstegen Spices & Sauces farmer cooperative Osacoop





© Pretaterra and Verstegen Spices & Sauces, Osacoop, 2020. Agroforesteria en Costa Rica: Deseños agroforestales con palma aceitera, cacao y vainilla. https://www.pretaterra.com/projetos-agrofloresta.

LIST OF PUBLICATIONS

Journal articles (peer reviewed)

Watteyn, C., Fremout, T., Karremans, A.P., Huarcaya, R.P., Azofeifa Bolaños, J.B., Reubens, B., Muys, B., 2020. Vanilla distribution modelling for conservation and sustainable cultivation in a joint land sparing / sharing concept. Ecosphere, 11(3), Art.No e03056. doi: 10.1002/ecs2.3056

Watteyn, C., Scaccabarozzi, D., Muys, B., Van der Schueren, N., Van Meerbeek, K., Amador, M.F G., Ackerman, J.D., Cedeno Fonseca, M., Chinchilla Alvarado, I.F., Reubens, B., Pillco Huarcaya, R., Cozzolino, S., Karremans, A.P., 2021. Trick or treat? Pollinator attraction in *Vanilla pompona* (Orchidaceae). Biotropica, 54 (1), 268-274. doi: 10.1111/btp.13034

Watteyn, C., Dejonghe, O., Van Hoyweghen, K., Bolanos, J.B A., Karremans, A.P., Vranken, L., Reubens, B., Muys, B., Maertens, M., 2021. Exploring farmer preferences towards innovations in the vanilla supply chain. Journal of Cleaner Production, 330, Art.No. 129831. doi: 10.1016/j.jclepro.2021.129831

Oral presentations

Watteyn, C., Fremout, T., Karremans, A., Pillco Huarcaya, R., Azofeifa Bolaños, J.B., Reubens, B., Muys, B. (2019). Playing hide and seek: Distribution of *Vanilla* crop wild relatives in Costa Rica. Presented at the III International Vanilla Congress, Veracruz, Mexico, March 5-8, 2019.

Watteyn, C., Scaccabarozzi, D., Muys, B., Van der Schueren, N., Van Meerbeek, K., Guizar Amador, M.F., Ackerman, J.D., Cedeño Fonseca, M.V., Chinchilla Alvarado, I.F., Reubens, B., Pillco Huarcaya, R., Cozzolino, S., Karremans, A.P. (2021). Trick or Treat? Pollinator attraction in *Vanilla pompona* (Orchidaceae). Presented at the IV International Vanilla congress, Cali, Colombia, December 1-4, 2021.

Thesis supervision and technical reports

Blaakman, K. 2022. Agroforestry vanilla from Costa Rica. Bachelor thesis and Research report International Food and Agribusiness, HAS University of Applied Sciences, Wageningen, The Netherlands.

Dejonghe, O. 2020. Farmers' interest in innovative approaches for sustainable vanilla production: A choice experiment in southwest Costa Rica. Master thesis, KU Leuven, Leuven, Belgium.

Van der Schueren, N. 2020. About flowers and bees: Wild Vanilla pollination by Euglossini in south-west Costa Rica. Master thesis, KU Leuven, Leuven, Belgium.

Pretaterra, Verstegen Spices & Sauces, Osacoop, 2020. Agroforesteria en Costa Rica: Deseños agroforestales con palma aceitera, cacao y vainilla. https://www.pretaterra.com/projetos-agrofloresta.