1 Tree species effects are amplified by clay content in acidic soils

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15 Abstract

16 The tree species composition of many forests in western and central Europe has changed considerably 17 over the last century, as large areas of mixed deciduous forests were replaced by conifer plantations. In 18 this study, we aim to evaluate whether conversion of mixed broadleaved forest to Norway spruce (Picea 19 abies) on the acidic soils of the Gaume forest (southern Belgium) affected soil quality in terms of soil acidity, exchangeable calcium and aluminium, humus type and earthworm communities, and determine 20 21 whether this effect is mitigated or amplified by an edaphic gradient in clay content. In this ancient 22 deciduous woodland, stands were partly converted from mixed deciduous forest to Norway spruce 23 monocultures 30 to 60 years ago. A twin-plot setup was established, where we sampled pairs of adjacent 24 deciduous and Norway spruce plots along a gradient in clay content varying between 3 and 34 %. This 25 design allowed to evaluate the effects of soil type and conversion independently. In the deciduous plots, 26 forest type ranged from mixed oak-hornbeam forest to oak-beech forest. The first has a higher clay 27 content, higher exchangeable calcium, mull humus type, low forest floor mass and the presence of 28 burrowing earthworms (endogeics), while the latter is characterised by high forest floor mass and 29 presence of only litter-dwelling earthworms (epigeics), respectively. Our results provide evidence that the 30 natural biogeochemical gradient converges to a narrow and acid range after conversion. When comparing 31 Norway spruce plots with adjacent broadleaved stands, topsoil pH, calcium concentrations and total 32 earthworm biomass were significantly lower, endogeic and epi-anecic earthworms were mostly absent, 33 and the exchangeable aluminium was significantly higher. Contrary to the current paradigm, the impact 34 of conversion in these acidic soils is largest for the stands with the highest clay content, where the larger exchange capacity allows greater accumulation of exchangeable aluminium when pH becomes sufficiently 35 36 low. These findings have important implications for forest management: for systems near a threshold in 37 soil process domain, it is important to realise that the sites with higher CEC and more favourable pH-38 values are the ones that will have larger trajectories and deteriorate the most upon acidification. Hence 39 restoring such stands into more natural deciduous or mixed forest may become increasingly difficult.

40 Highlights

- Forest conversion to Norway spruce narrows natural biogeochemical gradients on acidic soils
- Acidification trajectories after conversion were larger on richer forest sites
- Clay content amplifies acidification impact by a build-up of aluminium

44 Introduction

45 Temperate forests provide a multitude of provisioning, regulating and cultural ecosystem services. Forest management practices avoiding soil degradation are a precondition for a sustainable supply of these 46 47 services. One of the basic forest management decisions is the choice of overstory tree species, as it 48 triggers a chain of interactions between vegetation, soil chemical and physical properties and soil biological activity (Zinke, 1962; Van Breemen, 1993; Muys, 1995; Reich et al., 2005; De Schrijver et al., 49 50 2012). The tree species composition of many forests in western and central Europe has been changed 51 considerably since the 19th century: large areas of mixed deciduous forests were converted into conifer 52 plantations offering faster growth of highly demanded wood products (Klimo et al., 2000. Spiecker et al., 53 2004). Coniferous tree species are generally considered to negatively affect site quality, as their slow-54 decomposing litter, high interception of atmospheric pollutants and higher amounts of root exudates 55 have an acidifying effect on the forest soil (Binkley and Valentine, 1991; Ranger and Nys, 1994; Augusto 56 et al., 2002; Vesterdal et al., 2008). Due to this acidification, the availability of macro-nutrients and so-57 called base cations (i.e. potassium, calcium and magnesium) decreases, while proton and aluminium concentrations may rise to toxic levels with negative effects on tree health and productivity and above-58 59 and belowground biodiversity (Nordstrøm and Rundgren, 1974; Grossi and Brun, 1997; Wever et al., 2001; Eggleton et al., 2009; Calvaruso et al., 2011). 60

61 Soil type has been shown to modulate tree species effects, but contrasting views have emerged in 62 literature: some studies report a greater expression of species effects on poor sites, while others obtained inconclusive or opposite results (Finzi et al., 1998; Ste-Marie et al., 2007, Augusto et al., 2015; Raulund-63 64 Rasmussen & Vejre, 1995; Forrester et al., 2013). In most of these studies, soil texture was used as the 65 main proxy for site quality as it is relatively easy to measure or can be deduced from soil maps. Moreover, 66 in soils with similar lithologies, clay content is a measure for the pH-independent cation exchange capacity 67 (CEC) of a soil, and is largely unaffected by biotic drivers and management (Binkley and Fisher, 2013). 68 These studies however fail to consider what happens if the buffer capacity provided by the exchange 69 complex eventually runs out. Indeed, acid buffering capacity of soils is typically characterised by 70 biogeochemical equilibria with considerable pedogenic inertia (so-called 'buffer ranges' or 'soil process 71 domains'; Ulrich and Sumner, 1991; Vitousek and Chadwick, 2013), but thresholds are steep when one 72 buffering mechanism is exhausted and replaced by another (Chadwick and Chorover, 2001). Many 73 temperate forests at risk for acid-induced soil deterioration have a pH range close to the threshold 74 between the cation-exchange buffer domain and the aluminium-mediated buffer domain, corresponding to a transition from mull to moder/mor humus systems (Muys & Lust, 1992; Ponge, 2003; Andreetta et al., 2016). Studying tree species effects in forest systems near this pedogenic threshold can therefore provide vital new insights in the dynamic relationship between clay content and soil acidification upon conversion, provided that effects of tree and soil properties can be addressed independently. The latter is not straightforward as forest managers usually adapt their choice of overstory species to site fertility.

80 Moreover, soil biodiversity may have a much larger influence on soil quality than previously assumed 81 (Hale et al., 2005; Lavelle, 2006; Mueller et al., 2015; Filser et al., 2016), as the complexity of the soil food 82 web may dampen the effects of external drivers (Srivastava et al., 2009; Gesser et al., 2010; Morrien et 83 al., 2017) and burrowing soil fauna actively redistribute nutrients over the soil profile (Bohlen et al., 2004; 84 Briones, 2014; Lavelle et al., 2016). Earthworms are considered key ecosystem engineers for litter 85 decomposition and soil bioturbation that can be strongly affected by increased acidity (Muys & Granval, 86 1997; Schelfhout et al. 2017). Conversion-induced ecological shifts in earthworm communities are 87 therefore likely to affect the subsoil-topsoil-litter-layer continuum essential to forest nutrient cycles 88 (Ponge, 2003; Ste-Marie et al., 2007; Dawud et al., 2016).

89 Hence, in this study, we aim to evaluate the effect of conversion to Norway spruce (Picea abies) on soil 90 pH, available calcium, available aluminium, litter layer characteristics and earthworm species along a 91 gradient in soil clay content in the ancient deciduous forest of the Gaume (southern Belgium). Although 92 all soils of this vast forest complex are relatively sandy, a difference in marl content of the Jurassic parent 93 material resulted in a gradient of soil clay content varying from almost 0 to over 30%. Pedogenic 94 weathering has strongly depleted base cations in all soils: most have a pH and base saturation in the 95 aluminium-buffer range, while only the ones with the larger clay content remain in the cation-exchange 96 soil process domain prior to conversion (Verstraeten et al. 2013; Desie et al, 2017). Small patches of 97 Norway spruce have been introduced in this forest ca. four decades ago, allowing a twin-plot setup with 98 one plot in a mixed deciduous forest stand and one in an adjacent stand of Norway spruce. Twin-plots 99 were established along the lithological gradient, allowing to study conversion and soil effects 100 independently.

101 Materials and methods

102 Study site

103 The study area is part of a 200 km² forest complex in southern Belgium (Gaume region, centre 49° 37' N, 104 5° 33' E). It is considered an ancient forest, defined as being continuously forested since at least 1777 105 (Hermy et al., 1999). The deciduous forest is uneven-aged and dominated by Carpinus betulus (percentage 106 of total tree cover in the studied deciduous plots: 36%), Quercus robur (27%) and Fagus sylvatica (22%). 107 In this matrix of deciduous forest, patches of 2–15 ha were clear-cut and replanted with monoculture 108 stands of Norway spruce 30 to 50 years ago, creating small islands of coniferous forest in the deciduous 109 forest matrix. The elevation of the study area ranges between 250 and 360 m above sea level, the mean 110 annual temperature is 8.7°C, and the mean annual precipitation of 873 mm is evenly distributed 111 throughout the year. The site's parent material is a Jurassic calcareous sandstone (grès calcaire) with 112 variable marl content, which is part of a cuesta landscape adjacent to the Ardennes at the northern rim 113 of the Paris basin. Most profiles contain variable – albeit small – additions of Quaternary aeolian loess. 114 Soil texture therefore varies from sand to loam, and mainly differences in clay content determine the 115 intrinsic buffer capacity and CEC of the soils. Reference Soil Groups include Luvisols, Alisols and Cambisols 116 (WRB, 2015), are at present completely devoid of free carbonates and have low natural weathering to replenish base saturation (Bouezmarni et al., 2009). A total of 80 plots, consisting of 40 117 118 broadleaved/Norway spruce pairs, were selected along the gradient in clay content in a stepwise 119 approach. First, Norway spruce patches in the forest complex were identified on aerial photographs and 120 topographical maps, avoiding stands at the outer edges of the forest and taking into account information 121 from the soil texture map of the Service Public de Wallonie (2007) and the vegetation map of Dethioux 122 and Vanden Berghen (1966). Complemented with field observations, Norway spruce stands were 123 subsequently selected to have similar stand age, and to have a surrounding broadleaved matrix with 124 similar elevation and slope orientation maximizing comparability in soil type, forest history and original 125 tree species composition.

For each location, a twin-plot design was set out, with a square plot of 10 m x 10 m in each neighbouring stand (Figure 1). The centre of each plot was 30 m (approximately one tree height) from the border into both the deciduous and coniferous stands to balance the trade-off between minimizing spatial variation between plots and minimizing mutual influences between stands.

130 Data collection

131 In June 2009, the percent crown cover of all species in the tree layer was recorded. Soil samples were 132 taken at three depth intervals (0 - 5 cm, 10 - 20 cm and 25 - 35 cm) using a soil corer. For each depth 133 interval, four samples were taken in each stand (one randomly in each quadrant of each plot) and pooled 134 into a composite soil sample (Figure 1). The soil samples were dried at 40°C until constant mass and analysed for pH (extraction in 1 M KCl, 1:5 suspension, ion-specific electrode; ISRIC and FAO, 2002; ISO 135 136 10390:1994) at the three depths and exchangeable concentrations of Ca²⁺ and Al³⁺ (extraction in 0.1 M BaCl₂, 1:20 suspension and measurement with flame atomic absorption spectrophotometry, SpectrAA-137 220, Varian; Henderschot and Duquette, 1986; NEN-EN-ISO 11260:2011) for the 0-5 cm and 25-35 cm 138 139 layer. The soil texture of the 10 – 20 cm depth interval (expressed in percentages clay, sand and loam) 140 was determined by laser granulometry (Malvern Mastersizer S). The litter (L), fragmentation (F) and 141 humus (H) layer of the forest floor were collected separately in four squares of 20 x 20 cm² in each plot 142 (Jabiol et al., 1995; Figure 1). These samples were dried at 70°C until constant mass and their mass was determined. The total forest floor mass was the sum of the three layers. 143

144 The earthworm community was determined in thirty of the forty deciduous-spruce pairs, distributed to 145 span the entire gradient in soil clay content. Earthworms were sampled at two opposite corners of each 146 vegetation plot (Figure 1). The sampling was performed in three steps: first a 0.5 m² frame was put on the 147 ground in which the vegetation was carefully removed. From a smaller square of 0.1 m² positioned in the 148 middle of the larger (0.5 m²) square, the forest floor was collected and stored in plastic bags; the forest 149 floor outside that small square was removed. The earthworms in the collected forest floor were retrieved 150 by hand-sorting. Next, we poured 40 litre of mustard solution per plot (6 g mustard powder L⁻¹) in the 151 larger (0.5 m²) square (Bouché & Aliaga, 1986; Valckx et al., 2011; Schelfhout et al., 2017). Valckx et al., 152 2011). The earthworms were collected directly after emerging. Finally, a soil monolith (0.1 m² and 20 cm 153 deep) in the mustard treated area was thoroughly checked for earthworms by hand-sorting. All the 154 earthworms were first killed in 95 % ethanol and after a few hours transferred to a 5 % formalin solution 155 for fixation. After 3 days they were transferred to 95 % ethanol for further preservation and identification. 156 In the laboratory, the mass of the earthworms was determined with gut content and identified to species level following the nomenclature of Sims and Gerard (1999). Earthworm biomass was expressed as g m⁻² 157 158 ethanol-preserved mass. In the few cases that identification to species level was not possible, the 159 earthworms were identified at genus or ecological group level and were assigned pro rata to species level 160 (cf. Valckx et al., 2006).

161 Data analysis

162 To evaluate the initial quality of the deciduous forest plots before conversion to Norway spruce, overstory 163 composition, forest floor characteristics, soil properties and earthworm communities were correlated 164 with clay content (at 10-20 cm) and pH (0-5 cm) using Pearson's correlation in case of normality (Shapiro-165 Wilk test) and the presence of a linear relationship and Spearman rank correlation coefficients in other 166 cases. The first axis of a Detrended Correspondence Analysis (DCA) on the overstory vegetation in the 167 deciduous stands (canopy cover data) was used as a proxy for the overstory composition (Hill, 1979). Topsoil exchangeable calcium concentration was log-transformed to obtain a linear relation prior to 168 169 testing the correlation with clay content and pH. Subsequently Norway spruce plots were included in the 170 analysis as to evaluate conversion effects. After testing for normal distributions using the Shaparo-Wilk 171 test, we used non-parametric Wilcoxon signed-rank tests to evaluate whether the means of two sets of 172 paired measurements were significantly different. These pairwise tests were executed for pH-KCl, Al 173 concentration, Ca concentration (log transformed), forest floor mass and earthworm biomass. In addition, 174 we calculated the differences of these variables within pairs and correlated these differences with clay 175 content, to test whether clay content modulates acidification. All statistical analyses were performed with 176 R 3.3.2 (R Core Team, 2016) with 0.05 as the minimum significance level for all analyses.

177 Results

178 Site quality gradient in the deciduous forest matrix

179 The soil clay content varied between 3 and 34 % (Figure 2). In non-converted, deciduous stands this 180 gradient was correlated with overstory tree species composition, forest floor mass, soil acidity and 181 earthworm communities (Table 1). Overstory species composition varied from stands dominated by 182 Quercus robur and Fagus sylvatica in plots with low clay content to a species mixture containing Carpinus 183 betulus, Corylus avellana, Acer pseudoplatanus, Quercus robur, Fraxinus excelsior and Prunus avium in 184 plots with higher clay content. The total forest floor mass, the mass of the F layer and the H layer all 185 decreased with increasing clay content and pH-KCl. In 18 out of the 40 deciduous plots there was no H-186 layer present. The clay content of the soil was also significantly and positively correlated with the pH-KCl 187 of the mineral topsoil (0 - 5 cm) and with the exchangeable calcium concentration of the mineral topsoil 188 (0 - 5 cm), yet no correlation was found with exchangeable aluminium (Table 1 and Figure 2, closed 189 circles). Finally, both the endogeic and epigeic earthworm biomass were positively correlated with clay 190 content. Epigeic earthworm biomass ranged from 0.2 to 118.7 g m⁻² and endogeic earthworm biomass

191 ranged from 0 to 98.71g m⁻². Eleven species of earthworms were identified, belonging to the genera 192 Dendrobaena, Dendrodrilus, Lumbricus, Aporrectodea and Octolasion. The main epigeic species in the 193 broadleaved plots (by count) included Dendrodrilus rubidus, Lumbricus rubellus, and Dendrobaena 194 octaedra. The main endogeic species were Aporrectodea caliginosa and Aporrectodea rosea. The anecic 195 species Lumbricus terrestris was only found in one deciduous plot (Supplementary data, Table S1). All 196 these variables were also correlated with topsoil pH-KCl, which varied between 3.2 and 4.7 (Table 1). 197 Exchangeable aluminium varied in a unimodal pattern with pH-KCl, with a maximum at 3.6.

198 *Conversion effects*

199 The soil pH-KCl in the Norway spruce stands was significantly lower than in the deciduous stands for both 200 the 0 – 5 cm and the 10 – 20 cm soil layers ($p \le 0.001$; Figure 3). Correspondingly, the exchangeable 201 aluminium content of the topsoil (0-5 cm) and subsoil (25-35 cm) was significantly higher under Norway 202 spruce whereas exchangeable calcium concentrations were significantly lower ($p \le 0.001$ for topsoil and 203 $p \le 0.01$ for subsoil; Figure 3). In the subsoil layer (25 - 30 cm) no significant difference in pH-KCl is 204 observed, but even at this considerable depth, exchangeable aluminium was still significantly higher and 205 exchangeable calcium significantly lower under Norway spruce ($p \le 0.01$; Figure 3). The total forest floor 206 mass was between two and ten times higher in the Norway spruce stands compared to the deciduous 207 stands. The differences in biomass were significant for the L, F and H-layers separately, although most obvious and significant for the H-layer, which increased from an average of 152 g m⁻² under broadleaved 208 trees to an average of 1353 g m⁻² under Norway spruce (Figure 4). 209

210 Total earthworm biomass was significantly lower in the Norway spruce stands compared to the adjacent deciduous stands ($p \le 0.01$). The mean biomass decreased from 30.23g m⁻² (ranging from 0 to 200 g m⁻²) 211 212 for deciduous plots to 6.45g m⁻² (ranging from 0 to 25 g m⁻²) for Norway spruce plots. Epigeic earthworm 213 species were present in both coniferous and deciduous forest stands, however, their biomass was significantly lower in the Norway spruce stands, generally by a factor 1.5 - 5 (p ≤ 0.01 ; Figure 5 and 214 215 supplementary material S1). Endogeic earthworms were rare in the Norway spruce stands and their biomass was significantly lower compared to the deciduous stands ($p \le 0.01$; Figure 5). More specifically, 216 217 endogeic species were virtually absent in plots with topsoil pH-KCl values lower than 3.5 and their biomass 218 steeply increased in soils with pH-KCl higher than 4. Furthermore, conversion induced a shift in species 219 composition. To illustrate this, we compared the biomass between pairs for three common earthworm 220 species with distinct ecological preferences (Bouché, 1972; Muys and Granval, 1997; Haimi and Huhta, 221 1990; Eisenhauer et al., 2008), i.e. Dendrobaena octaedra, Lumbricus rubellus and Aporrectodea

caliginosa (supplementary material, Table S1 and Figure S2). The biomass of *A. caliginosa*, an endogeic earthworm species and of *L. rubellus*, a mesophile epi-anecic species (epigeic with large litter decomposing capacity and some soil burrowing activity), is significantly higher in deciduous stands compared to adjacent Norway spruce stands, with a total collapse of the population of *A. caliginosa* under spruce. The acid tolerant strictly epigeic species *D. octaedra* was less affected by conversion to Norway spruce.

228 Modulation by clay content

229 Of the differences described above, a significant correlation was found between clay content and the pH 230 change after conversion, as measured by the pairwise difference in pH of the topsoil between deciduous 231 and Norway spruce plots (Figure 2). A larger increase in topsoil pH difference can be observed with 232 increasing clay content (Spearman's rank coefficient = +0.34 with $p \le 0.05$, Figure 3 and Figure 2a, right 233 panel). The differences in aluminium and calcium concentrations within the spruce-deciduous pair are 234 also significantly correlated with clay content (Figure 3). Twin-plots with a higher clay content have higher 235 exchangeable aluminium content after conversion, and experience a significantly larger increase in available aluminium compared to plots with a lower clay content (Spearman's rank coefficient = -0.63 with 236 237 $p \le 0.001$; Figure 3 and Figure 2b).

The exchangeable calcium concentration ranges from 51.7 to 1736.6 mg kg⁻¹ in deciduous plots whereas a narrower low range of 35.5 to 482.4 mg kg⁻¹ was observed under Norway spruce (Figure 2c, left panel), with low values for all plots, although the loss is significantly higher in the plots with the higher clay content (Spearman's rank coefficient = +0.39 with p \leq 0.05; Figure 3 and Figure 2c, right panel).

242 In deeper soil layers (0 - 10 cm and 25 - 30 cm) conversion trends were more subtle and differences in 243 pH, aluminium and calcium concentration within the deciduous-spruce pair could not be correlated with 244 clay content. In the forest floor the difference in mass of the fragmentation-layer (F-layer) between 245 deciduous and Norway spruce plots was significantly correlated with clay content (Figure 3). This implies 246 that the increase in F-layer after conversion is larger for plots with higher clay content. Finally, clay content 247 also modulates the biomass of epigeic and endogeic earthworms ($p \le 0.01$; Figure 4), with the difference in biomass between the deciduous plot and the Norway spruce plot increasing with increasing clay 248 249 content.

250 Discussion

The twin-plot design with plot pairs of original broadleaved and newly established Norway spruce in the Gaume forest has proven to be a solid research platform for evaluation of forest conversion effects (Verstraeten et al., 2013). The innovative strength of the set-up lies in the lithological gradient across the study site, which allows evaluating the interaction of forest conversion on aboveground- and belowground forest ecosystem functioning with the edaphic site quality, in this case the clay content.

The difference in soil type induces natural diversity in the deciduous stands, as the plots with the highest clay content have a higher pH, lower exchangeable aluminium, higher available calcium, lighter forest floors with significantly smaller F and H layers and support more demanding tree species and bigger and more diverse earthworm communities. In unconverted plots, the clay content acts as a mitigating factor for pedogenic depletion of base cations in the Jurassic parent material, as the bigger exchange capacity retains more nutrients (in this case calcium). Desie et al. (2017) confirmed that the clay-rich plots correspond to the exchange buffer range (Ulrich and Sumner, 1991; Vitousek and Chadwick, 2013).

263 This effect however completely shifts after conversion: although their pH is still slightly higher than in the 264 sandier plots, soils with a higher clay content have the same low available calcium levels, but retain 265 significantly more exchangeable aluminium. The higher potential of the clay-rich soils to retain base 266 cations implies that they can also store much larger loads of exchangeable aluminium, to levels of 300-500 mg Al kg⁻¹ that are toxic to many plant roots and soil species (Van Gestel and Hoogerwerf, 2001; De 267 268 Wit et al., 2010; Schelfhout et al., 2017). Effects on soil communities after conversion are therefore most 269 drastic in the clay rich soils, i.e. on the endogeic and epi-anecic earthworms which are virtually absent in 270 the Norway spruce stands. Epigeic species are less affected but also decline significantly upon increasing 271 aluminium loads, which is in line with their ecological limits reported in literature (Muys and Lust, 1992; 272 Muys and Granval, 1997; Schelfhout et al., 2017). The effects of the loss of these important bioturbation 273 agents on the soil's decomposition cycles are illustrated by a significant increase in the thickness of the F-274 layer, and the emergence of a H-layer. Albeit very variable in Norway spruce stands, the total mass of this 275 layer is consistently low in deciduous plots. Increases in the F and H layers correspond to more 276 decomposition in the litter layer opposed to the soil (Ponge, 2013), further reducing availability of base 277 cations in the soil to a general low level: as calcium is an essential plant nutrient, a delayed return from 278 the forest floor causes rapid depletion of nearly all remaining plant available calcium from the soil (Figure 279 2c). Our observations are in line with the biotic feedback loop described in the literature where low litter 280 quality and resulting acidification lowers earthworm biomass and bioturbation (Nordstrøm and Rundgren,

1974; Muys et al., 1992; Edwards, 2004; Reich et al., 2005, Hobbie et al., 2006), which in turn enhances
the forest floor accumulation and aggravates acidification (de Vries and Breeuwsma, 1985; Nilsson et al.,
1982). A scope for further research is to investigate how the conversion from broadleaved forest to conifer
monocultures affects other below-ground communities, such as bacteria, fungi and micro-arthropods.

285 Our study therefore confirms the current consensus in literature that Norway spruce is considered a 286 species with strong ecological effects and high acidifying potential especially in the upper soil layers (e.g. 287 Tamm and Hallbäcken, 1986; Augusto et al., 1998; Hagen-Thorn et al., 2004; Ranger and Nys, 1994; De 288 Schrijver et al., 2007 and 2012, Augusto et al., 2015, Cremer & Prietzel, 2017). Yet it challenges the general 289 paradigm that conversion effects are more severe in poorer soils (e.g. Augusto et al., 2002; Ste-Marie et 290 al., 2007). In fact we found the opposite: for acidic soils on parent materials with low natural weathering, 291 clay content amplifies tree species effects once the acid buffering potential of the clay-associated CEC 292 eventually is exhausted and the exchange complex starts accumulating toxic aluminium cations. Chadwick 293 and Chorover (2001) describe a similar mechanism when crossing the pedogenic threshold in acid 294 buffering capacity between the 'cation exchange buffering domain' and the 'aluminium buffering domain' 295 in soils with progressive pedogenic weathering. Ponge (2003) describes a shift to a more primitive and 296 aluminium rich carbon cycle in acid soils, but tree species are generally not considered as a potential driver 297 that can topple a system from one buffering mechanism to another. Stronger aluminium retention upon 298 soils with higher CEC is a common concept in agricultural soils (Driessen et al., 2001) but largely ignored 299 when studying acidification in forests. In the Gaume, the natural geochemical diversity of the stands 300 narrowed considerably after conversion, with all Norway spruce plots located in a very narrow and acid 301 pH-KCl range of 3.0 to 3.7. Desie et al. (2017) confirmed that all converted plots are part of the aluminium 302 buffering domain.

303 These findings have important implications for forest management: for systems near a threshold between 304 the cation-exchange buffer domain and the aluminium-mediated buffer domain, it is important to realise 305 that the sites with the best soils, higher CEC and more favourable pH-values are actually the ones that will 306 have larger trajectories and deteriorate the most upon acidification. In terms of biogeochemical diversity, 307 conversion to Norway spruce made the stands in the Gaume forest not only more acid but also more 308 uniform, potentially harming biodiversity in the long run as they all converged to the same narrow pH 309 range (Verstraeten et al., 2013). Moreover, aluminium has a strong preferential retention on the soil's 310 exchange complex (Driessen et al., 2001; Binkley and Fisher, 2013). Hence, as both clay content and 311 biological feedback loops amplify the acidifying tree species effect of Norway spruce conversion in the Gaume forest, restoring such coniferous plantations into more natural deciduous or mixed stands may
 become increasingly difficult, requiring integrated soil restoration efforts, combining tree species change,
 nutrient gifts and earthworm reintroduction (Muys et al., 2003).

315 Conclusion

316 This study aimed at evaluating the effect of conversion from ancient deciduous forest to Norway spruce 317 plantation in the Gaume forest (Wallonia, Belgium) on soil biogeochemistry, litter layer characteristics and 318 earthworm communities. Our results provide evidence that the natural biogeochemical gradient, 319 essential for the forest's extraordinary biodiversity, converges to a narrow and acid range after 320 conversion. Contrary to the current paradigm that clay content mitigates acidification, the impact of the 321 conversion on the forest floor-topsoil-subsoil continuum was larger in the stands with the highest clay 322 content. In the acidic soils of the Gaume, the larger exchange capacity allows for a larger accumulation of 323 exchangeable aluminium when pH becomes sufficiently low. This process is enhanced by a biotic feedback 324 loop involving a decline of earthworm biomass and a shift to acid-tolerant, epigeic species. In turn, the 325 decrease of bioturbation potential further enhances the conversion effect. Hence, massive overstory 326 conversion may threaten biodiversity conservation and the potential for site restoration in the long run.

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- 502 Table 1. Correlations (Spearman rho correlation coefficient) between variables describing ecosystem components
- for story vegetation, forest floor, topsoil and earthworms), clay content (at 10 20 cm) and topsoil pH (at 0 5
 cm) in unconverted deciduous plots. FFM: forest floor mass. Topsoil: 0 5 cm layer. Significance: * P ≤ 0.05; ** P ≤
- **0.01**; *** P ≤ 0.001.

Ecosystem component	Tested variable	Clay content of the	Topsoil pH-KCl
		soil	
Overstory vegetation	First DCA axis overstory	0.56**	0.87***
Forest floor	Total FFM	- 0.55**	- 0.79***
	FFM - litter layer	NS	NS
	FFM - fragmentation layer	-0.54**	-0.71***
	FFM – humus layer	-0.52**	-0.72***
Topsoil	рН-КСІ	0.65***	
	Log (exchangeable Ca)	0.58***	0.84***
	Exchangeable Al	NS	NS
Earthworm community	Endogeic earthworm biomass	0.54**	0.84***
	Epigeic earthworm biomass	0.49**	0.59***

508 Figure captions

Figure 1: Scheme of the paired plot set-up in the Gaume forest. Each twin-plot consists of one 10x10m sampling square in the broadleaved matrix and one in the adjacent Norway spruce monoculture. In each square, four subsamples were taken randomly in each quadrant of each square for soil analysis, litter layer characteristics and earthworm sampling.

513 Figure 2. Relation between soil clay content (at 10-20 cm) and topsoil pH (0 – 5 cm) (a), aluminium (b) and calcium 514 (c) concentrations for deciduous and Norway spruce plots. Left panels show raw data for spruce (black dots) and 515 deciduous (white dots)) and right panels show the pairwise differences between spruce and deciduous plots.

Figure 3. Comparison of pH-KCl, exchangeable calcium and aluminium concentrations in different soil layers of deciduous (light grey) and Norway spruce (dark grey) stands (number of pairs = 40). Significant differences are shown (Wilcoxon signed-rank test): * $P \le 0.05$; ** $P \le 0.01$; *** $P \le 0.001$. If the pairwise difference between deciduous and spruce plots (calculated per twin-plot as the value of the deciduous plot minus the value for the spruce plot) is significantly modulated by clay content, the correlation coefficient of clay with the pairwise difference variable is indicated (in red).

Figure 4. Comparison of the biomass of the forest floor layers (L: litter layer; F: fragmentation layer; H: humus layer) of the deciduous (light grey) and Norway spruce (dark grey) stands. Significant differences are shown (Student t-test and Wilcoxon signed-rank test): * $P \le 0.05$; ** $P \le 0.01$; *** $P \le 0.001$. If the pairwise difference between deciduous and spruce plots (calculated per twin-plot as the value of the deciduous plot minus the value for the spruce plot) is significantly modulated by clay content the correlation coefficient of clay with the pairwise difference variable is indicated (red).

Figure 5. Comparison of the epigeic and endogeic earthworm biomass of the deciduous (light grey) and Norway spruce (dark grey) plots. Significant differences are shown (Wilcoxon signed-rank test): * $P \le 0.05$; ** $P \le 0.01$; *** P ≤ 0.001 . If the pairwise difference between deciduous and spruce plots (calculated per twin-plot as the value of the deciduous plot minus the value for the spruce plot) is significantly modulated by clay content the correlation

532 coefficient of clay with the pairwise difference variable is indicated (red).

534 Supplementary material

535	Table S1: List of earthworm biomass (g m ⁻²) per species or genus over all paired plots; D indicating deciduous plots
536	and S indicating Norway spruce plots. The pairs are ranked based on their clay content.

- 537 Figure S2: Comparison of the earthworm biomass of the deciduous (light grey) and Norway spruce (dark grey)
- 538 stands for selected indicator earthworm species: *D. octaedra, L. rubellus, L. castaneus and A. caliginosa*.

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572 Figure 5



