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

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# **Abstract**

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

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

## **Introduction**

 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 services. One of the basic forest management decisions is the choice of overstory tree species, as it 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., 2012). The tree species composition of many forests in western and central Europe has been changed 51 considerably since the 19<sup>th</sup> century: large areas of mixed deciduous forests were converted into conifer plantations offering faster growth of highly demanded wood products (Klimo et al., 2000. Spiecker et al., 2004). Coniferous tree species are generally considered to negatively affect site quality, as their slow- decomposing litter, high interception of atmospheric pollutants and higher amounts of root exudates have an acidifying effect on the forest soil (Binkley and Valentine, 1991; Ranger and Nys, 1994; Augusto et al., 2002; Vesterdal et al., 2008). Due to this acidification, the availability of macro-nutrients and so- 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- and belowground biodiversity (Nordstrøm and Rundgren, 1974; Grossi and Brun, 1997; Wever et al., 2001; Eggleton et al., 2009; Calvaruso et al., 2011).

 Soil type has been shown to modulate tree species effects, but contrasting views have emerged in 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- Rasmussen & Vejre, 1995; Forrester et al., 2013). In most of these studies, soil texture was used as the main proxy for site quality as it is relatively easy to measure or can be deduced from soil maps. Moreover, in soils with similar lithologies, clay content is a measure for the pH-independent cation exchange capacity (CEC) of a soil, and is largely unaffected by biotic drivers and management (Binkley and Fisher, 2013). These studies however fail to consider what happens if the buffer capacity provided by the exchange complex eventually runs out. Indeed, acid buffering capacity of soils is typically characterised by biogeochemical equilibria with considerable pedogenic inertia (so-called 'buffer ranges' or 'soil process domains'; Ulrich and Sumner, 1991; Vitousek and Chadwick, 2013), but thresholds are steep when one buffering mechanism is exhausted and replaced by another (Chadwick and Chorover, 2001). Many temperate forests at risk for acid-induced soil deterioration have a pH range close to the threshold 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.

 Moreover, soil biodiversity may have a much larger influence on soil quality than previously assumed (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 al., 2017) and burrowing soil fauna actively redistribute nutrients over the soil profile (Bohlen et al., 2004; 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, 1997; Schelfhout et al. 2017). Conversion-induced ecological shifts in earthworm communities are therefore likely to affect the subsoil-topsoil-litter-layer continuum essential to forest nutrient cycles (Ponge, 2003; Ste-Marie et al., 2007; Dawud et al., 2016).

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

## **Materials and methods**

#### *Study site*

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

126 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.

*Data collection* 

 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  $(0 - 5 \text{ cm}, 10 - 20 \text{ cm}$  and  $25 - 35 \text{ cm})$  using a soil corer. For each depth interval, four samples were taken in each stand (one randomly in each quadrant of each plot) and pooled 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 136 10390:1994) at the three depths and exchangeable concentrations of  $Ca^{2+}$  and  $Al^{3+}$  (extraction in 0.1 M 137 BaCl<sub>2</sub>, 1:20 suspension and measurement with flame atomic absorption spectrophotometry, SpectrAA- 220, Varian; Henderschot and Duquette, 1986; NEN-EN-ISO 11260:2011) for the 0 – 5 cm and 25 – 35 cm layer. The soil texture of the 10 – 20 cm depth interval (expressed in percentages clay, sand and loam) 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 cm<sup>2</sup> in each plot (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.

 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  $m<sup>2</sup>$  frame was put on the 147 ground in which the vegetation was carefully removed. From a smaller square of 0.1 m<sup>2</sup> positioned in the middle of the larger (0.5 m²) square, the forest floor was collected and stored in plastic bags; the forest 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 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 m<sup>2</sup> and 20 cm deep) in the mustard treated area was thoroughly checked for earthworms by hand-sorting. All the earthworms were first killed in 95 % ethanol and after a few hours transferred to a 5 % formalin solution for fixation. After 3 days they were transferred to 95 % ethanol for further preservation and identification. In the laboratory, the mass of the earthworms was determined with gut content and identified to species 157 level following the nomenclature of Sims and Gerard (1999). Earthworm biomass was expressed as g m<sup>-2</sup> ethanol-preserved mass. In the few cases that identification to species level was not possible, the earthworms were identified at genus or ecological group level and were assigned pro rata to species level (cf. Valckx et al., 2006).

# *Data analysis*

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

#### **Results**

# *Site quality gradient in the deciduous forest matrix*

 The soil clay content varied between 3 and 34 % (Figure 2). In non-converted, deciduous stands this gradient was correlated with overstory tree species composition, forest floor mass, soil acidity and earthworm communities (Table 1). Overstory species composition varied from stands dominated by *Quercus robur* and *Fagus sylvatica* in plots with low clay content to a species mixture containing *Carpinus betulus, Corylus avellana*, *Acer pseudoplatanus, Quercus robur, Fraxinus excelsior* and *Prunus avium* in plots with higher clay content. The total forest floor mass, the mass of the F layer and the H layer all decreased with increasing clay content and pH-KCl. In 18 out of the 40 deciduous plots there was no H- layer present. The clay content of the soil was also significantly and positively correlated with the pH-KCl of the mineral topsoil (0 – 5 cm) and with the exchangeable calcium concentration of the mineral topsoil (0 – 5 cm), yet no correlation was found with exchangeable aluminium (Table 1 and Figure 2, closed 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  $m<sup>-2</sup>$  and endogeic earthworm biomass

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

# *Conversion effects*

 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 aluminium content of the topsoil (0 – 5 cm) and subsoil (25 – 35 cm) wassignificantly higher under Norway 202 spruce whereas exchangeable calcium concentrations were significantly lower ( $p \le 0.001$  for topsoil and 203 p  $\leq$  0.01 for subsoil; Figure 3). In the subsoil layer (25 – 30 cm) no significant difference in pH-KCl is 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 mass was between two and ten times higher in the Norway spruce stands compared to the deciduous stands. The differences in biomass were significant for the L, F and H-layers separately, although most 208 obvious and significant for the H-layer, which increased from an average of 152 g m<sup>-2</sup> under broadleaved 209 trees to an average of 1353 g m<sup>-2</sup> under Norway spruce (Figure 4).

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

## *Modulation by clay content*

229 Of the differences described above, a significant correlation was found between clay content and the pH change after conversion, as measured by the pairwise difference in pH of the topsoil between deciduous 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  $\leq$  0.05, Figure 3 and Figure 2a, right panel). The differences in aluminium and calcium concentrations within the spruce-deciduous pair are also significantly correlated with clay content (Figure 3). Twin-plots with a higher clay content have higher 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  $p \le 0.001$ ; Figure 3 and Figure 2b).

238 The exchangeable calcium concentration ranges from 51.7 to 1736.6 mg kg $^{-1}$  in deciduous plots whereas 239 a narrower low range of 35.5 to 482.4 mg kg<sup>-1</sup> 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 241 content (Spearman's rank coefficient = +0.39 with  $p \le 0.05$ ; Figure 3 and Figure 2c, right panel).

 In deeper soil layers (0 – 10 cm and 25 – 30 cm) conversion trends were more subtle and differences in pH, aluminium and calcium concentration within the deciduous-spruce pair could not be correlated with clay content. In the forest floor the difference in mass of the fragmentation-layer (F-layer) between deciduous and Norway spruce plots was significantly correlated with clay content (Figure 3). This implies 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 content.

## **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).

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

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

 These findings have important implications for forest management: for systems near a threshold between the cation-exchange buffer domain and the aluminium-mediated buffer domain, it is important to realise that the sites with the best soils, higher CEC and more favourable pH-values are actually the ones that will have larger trajectories and deteriorate the most upon acidification. In terms of biogeochemical diversity, conversion to Norway spruce made the stands in the Gaume forest not only more acid but also more uniform, potentially harming biodiversity in the long run as they all converged to the same narrow pH range (Verstraeten et al., 2013). Moreover, aluminium has a strong preferential retention on the soil's exchange complex (Driessen et al., 2001; Binkley and Fisher, 2013). Hence, as both clay content and 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).

# **Conclusion**

 This study aimed at evaluating the effect of conversion from ancient deciduous forest to Norway spruce plantation in the Gaume forest (Wallonia, Belgium) on soil biogeochemistry, litter layer characteristics and earthworm communities. Our results provide evidence that the natural biogeochemical gradient, essential for the forest's extraordinary biodiversity, converges to a narrow and acid range after conversion. Contrary to the current paradigm that clay content mitigates acidification, the impact of the conversion on the forest floor-topsoil-subsoil continuum was larger in the stands with the highest clay content. In the acidic soils of the Gaume, the larger exchange capacity allows for a larger accumulation of exchangeable aluminium when pH becomes sufficiently low. This process is enhanced by a biotic feedback loop involving a decline of earthworm biomass and a shift to acid-tolerant, epigeic species. In turn, the decrease of bioturbation potential further enhances the conversion effect. Hence, massive overstory 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**
- 503 **(overstory vegetation, forest floor, topsoil and earthworms), clay content (at 10 – 20 cm) and topsoil pH (at 0 – 5**
- 504 **cm) in unconverted deciduous plots. FFM: forest floor mass. Topsoil: 0 - 5 cm layer. Significance: \* P ≤ 0.05; \*\* P ≤**   $0.01$ ; \*\*\* P  $\leq$  0.001.



# **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 sub- samples were taken randomly in each quadrant of each square for soil analysis, litter layer characteristics and earthworm sampling.

 Figure 2. Relation between soil clay content (at 10-20 cm) and topsoil pH (0 – 5 cm) (a), aluminium (b) and calcium (c) concentrations for deciduous and Norway spruce plots. Left panels show raw data for spruce (black dots) and 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 518 (Wilcoxon signed-rank test): \* P  $\leq$  0.05; \*\* P  $\leq$  0.01; \*\*\* P  $\leq$  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 ≤ 0.05; \*\* P ≤ 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 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 ≤ 0.05; \*\* P ≤ 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

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

# **Supplementary material**

















Figure 5



