



Contents lists available at ScienceDirect

## Organic Geochemistry

journal homepage: [www.elsevier.com/locate/orggeochem](http://www.elsevier.com/locate/orggeochem)

# Disentangling the effects of parent material and litter input chemistry on molecular soil organic matter composition in converted forests in Western Europe



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## ARTICLE INFO

## Article history:

Received 6 February 2019

Received in revised form 1 April 2019

Accepted 27 May 2019

Available online 29 May 2019

## Keywords:

SOM

SOC

THM-GC-MS

Carbon stocks

Lignin

Cutin

Suberin

## ABSTRACT

By storing carbon in the soil, forests contribute to climate change mitigation. Edaphic (soil-related) factors, such as soil pH, as well as tree species affect forest carbon cycles, but are difficult to disentangle. We studied how conversion of deciduous stands to mono-culture spruce plantations affected the soil organic matter (SOM) composition along a lithological gradient in the Mullerthal (Luxembourg) and Gaume (south-east Belgium) regions. Parent materials in these regions range from decalcified sands to calcareous marls. A twin plot setup of adjacent deciduous and coniferous stands on the same parent material was used to evaluate the effect of edaphic factors versus litter input differences on SOM composition and soil organic carbon (SOC) stocks. Lignin and cutin/suberin molecular proxies were identified with thermally assisted hydrolysis and methylation (THM), to distinguish litter sources (coniferous vs deciduous and leaf litter vs roots) in the studied stands. In this study, SOC stocks were influenced more by parent material than by forest type. Lignin yield, composition and degradation state were influenced both by litter input chemistry and edaphic context. There appear to be important interaction effects between the two, as the relative importance of parent material and litter quality was site specific. We therefore advise that carbon stock models include data on both vegetation history as well as edaphic context.

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## 1. Introduction

The soil is the most important sink of terrestrial carbon, storing at least 1500 Pg of organic carbon as soil organic matter (SOM) (Batjes, 2014). Forest soils store a disproportionate amount of carbon compared to other land uses, with 40–70% of all soil carbon in the world being stored in forest soils (Lal, 2005; Stocker et al., 2013). Therefore, increasing forest SOM stocks is proposed as important measure to contribute to the mitigation of climate change (Stocker et al., 2013).

Many studies indicate the role of trees in climate change mitigation (D'Amato et al., 2011; Naudts et al., 2016), highlighting the effect of conversion of broadleaved forests to coniferous plantations for commercial purposes. Forest conversion affects both

carbon stocks and the rate at which carbon accumulates, however these seem to be inversely correlated (D'Amato et al., 2011). Forest conversion also changes forest structure, thereby affecting the exchange of water and energy with the atmosphere (Naudts et al., 2016). Naudts et al. (2016) found a temperature increase caused by species conversion. This already shows that not all forest conversion contributes to climate change mitigation. Forest management is further complicated by the potential incompatibility between mitigation and adaption measures (D'Amato et al., 2011). Although there is ample evidence that forest management influences SOM quantity (Jandl et al., 2007), the relationships between tree species, parent material, management decisions and forest SOM quantity are not sufficiently clear to design optimal management, planning and policy plans that are effective in mitigating the consequences of climate change (Naudts et al., 2016).

There is increasing evidence that the effects of tree species on SOM stocks and their allocation to different soil horizons are

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site-specific and relate to pedogenic gradients (from pedogenesis: soil formation or soil development), due to complex (and often non-linear) interactions between biotic and abiotic drivers in the soil (Cremer et al., 2016; Spielvogel et al., 2016). However, it remains problematic to disentangle the effects of biotic and abiotic factors on SOM, as they are often confounded (Kirschbaum, 2000). Parent material, through its influence on many soil properties (such as pH and texture), is an important abiotic factor for SOM turnover and properties (Six and Jastrow, 2002; Luo et al., 2017). For instance, parent material directly affects texture and soil type, which influences sorption and desorption of SOM, and thereby indirectly affects SOM turnover (Schmidt et al., 2011). In line with this, Hagedorn et al. (2001) found a larger C storage for acidic loam compared to calcareous sand, despite less biomass being produced in the acidic loam soil. This was likely caused by unfavourable conditions for mineralization (low pH) and a greater physico-chemical protection of C by clay and oxides in the acidic loam (Hagedorn et al., 2001).

Although vegetation, through litter input, is an important determining factor of the molecular SOM composition in maritime temperate forests (Vancampenhout et al., 2010), assessments of forest conversion effects on SOM have mainly focused on total carbon stocks. SOM composition, however, is an important aspect of forest soil carbon dynamics, as it directs the substrates available for soil fauna and micro-organisms (Kögel-Knabner, 2002) and therefore affects their distribution (Kooijman and Cammeraat, 2010). Moreover, the molecular SOM composition influences organic compound solubility and organo-mineral interactions (Six and Paustian, 2014), thereby affecting the physical accessibility of SOM for decomposers. Lastly, the molecular SOM composition encompasses important clues about the SOM origin and the level of processing by soil organisms, which are important proxies for soil ecosystem functioning (Gleixner, 2013).

Lignin compounds are commonly used to distinguish between gymnosperm and angiosperm input to SOM (Kögel-Knabner, 2002). Lignin is a complex biopolymer of vascular plants with three main building blocks; *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol (Hedges and Mann, 1979; Thevenot et al., 2010). Coniferyl alcohol and sinapyl alcohol can be traced back to guaiacol, syringol and their derivatives, respectively (Thevenot et al., 2010). Gymnosperms such as spruce, contain only guaiacyl-lignin, whereas angiosperms such as beech contain both guaiacyl- and syringyl-lignin (Nierop and Filley, 2008). Moreover, lignin side-chain degradation is a useful indicator of microbial alteration of lignin. Higher lignin side-chain degradation indicates greater microbial alteration of lignin, and thus may indicate greater below-ground dynamics.

Cutin, a bio-polyester, and suberin, a polymer with polyester domains, are used as molecular proxies of above- (cutin) and below-ground (suberin) biomass (Nierop, 2001; Nierop and Verstraten, 2004), and can potentially show how forest conversion affects the relative importance of litter and root carbon as inputs of SOM. Both are structural components in higher plants (Kolattukudy, 1980): cutin is mainly found in the aerial parts of plants, such as leaf surfaces, suberin is a component of underground plant organs, such as roots and tubers, and of wound surfaces and bark. Chemical analysis has shown that suberin can be distinguished from cutin by having a high content of phenolics and by having a high proportion of  $\omega$ -hydroxy acids, the corresponding dicarboxylic acids, and fatty acids as well as alcohols with more than 18 carbon atoms (Kolattukudy, 1980). Cutin compounds can be divided in a C<sub>16</sub> and a C<sub>18</sub> family of (poly)hydroxy and epoxy fatty acids. However, as C<sub>16</sub> and C<sub>18</sub> hydroxy acids can be found in both cutin and suberin, vegetation material has to be analysed first to enable the use of cutin and suberin as molecular proxies of above- and below-ground biomass (Nierop, 2001; Nierop and Verstraten, 2004).

This paper assesses how SOM quantity and composition are affected by conversion from broadleaved species to spruce monocultures in the temperate climate zone and evaluates how such changes are affected by differences in parent material and edaphic (soil related) factors. For this purpose, twin plots were laid out across a gradient in edaphic conditions in the Gaume region in Belgium and in the Mullerthal region in Luxembourg. Each twin plot consists of a plot in a deciduous stand and one in an adjacent coniferous stand on the same soil type (Verstraeten et al., 2018). Twin plots were equally divided over a 'sandy' and a 'loamy' substrate group to disentangle the effects of litter input chemistry and parent material on SOM composition. The twin plots have a known and similar vegetation history including a partial conversion of deciduous forest to mono-culture spruce plantations. The molecular SOM composition was analysed with thermally assisted hydrolysis and methylation (THM) (Nierop et al., 2005). We used soil carbon stocks, lignin, cutin and suberin to answer the following questions: (i) What is the relative importance of litter input chemistry and parent material on the molecular SOM characteristics? (ii) How is SOM composition influenced by a change in the litter input composition and does a legacy effect remain several decades after forest conversion? and (iii) what is the relative importance of root and litter input on the SOM composition in converted and non-converted forest soils?

## 2. Materials and methods

### 2.1. Site description, twin plots and sampling

The Gaume region (south-east Belgium) harbours a large, old-growth forest complex and has a sub-Atlantic maritime climate (mean annual temperature: 8.7 °C; mean annual precipitation: 873 mm) (KMI, 2014). Oak (*Quercus robur*), beech (*Fagus sylvatica*) and hornbeam (*Carpinus betulus*) dominate the deciduous forests. Sporadically some sycamore (*Acer pseudoplatanus*) occurs. Coniferous forest patches of 1–10 ha of spruce monocultures (*Picea abies*) were planted into the deciduous matrix about half a century ago. Although the range in pH values of Gaume plots (3.5–4.5) is relatively small compared to Mullerthal plots, Verstraeten et al. (2013, 2018) showed a high spatial variation in tree species, total carbon stocks, nutrient availability and earthworm and microbial communities. Parent material in the Gaume belongs to two intercalating geologic formations, namely the formation of Arlon, consisting of sandy marl and clayey limestone, and the formation of Luxembourg, consisting of sandstone and sandy limestone (Bouezmarni et al., 2009). Soils on the formation of Arlon therefore have a higher clay content than those formed on the formation of Luxembourg. Combined with a small and variable input of aeolian loess, soil texture ranges from sand to sandy loam (Verstraeten et al., 2018).

The Mullerthal region (mid-east Luxembourg) has a temperate climate (mean annual temperature: 8 °C; mean annual precipitation: 740 mm) (Meteo Luxembourg, 2014) and deciduous forests in this region are dominated by beech and hornbeam intermixed with oak, and some large-leaved linden (*Tilia platyphyllos*) on limestone (Kooijman and Martinez-Hernandez, 2009). Most Mullerthal plots hold old-growth forests similar to those of the Gaume. However, terrace agriculture may have occurred in Roman times in twin plot 12 (Table 1), followed by land abandonment and spontaneous reforestation. Twin plots 7 and 9 may have been reforested in the large reforestation that took place around 1850, with no evidence of ploughing. Forest patches of spruce were planted in the second half of the last century as in the Gaume. The pH in the Mullerthal twin plots ranged from 3–5 to 6–7: earlier research showed an increase in total carbon stocks in the A-horizon and a shift in

**Table 1**  
Lithology, soil type and substrate group of the twin plots.

Plot	Study area	Substrate group	Parent material	Soil type
1	Gaume	Sandy	Sandstone <sup>a</sup>	Luvisol
2	Gaume	Sandy	Sandstone <sup>a</sup>	Dystric Arenosol
3	Gaume	Sandy	Sandstone <sup>a</sup>	Dystric Cambisol (Loamic)
4	Gaume	Loamy	Sandstone <sup>a</sup>	Leptic Regosol
5	Gaume	Loamy	Sandstone <sup>a</sup>	Cambisol Loamic (Humic)
6	Gaume	Loamy	Sandstone <sup>a</sup>	Cambisol (Loamic)
7	Mullerthal	Sandy	Sandstone <sup>b</sup>	Dystric Arenosol
8	Mullerthal	Sandy	Sandstone <sup>b</sup>	Leptic Dystric Regosol
9	Mullerthal	Sandy	Sandstone <sup>b</sup>	Leptic Dystric Regosol
10	Mullerthal	Loamy	Marls <sup>c</sup>	Leptic Dystric Stagnosol
11	Mullerthal	Loamy	Loess on marls <sup>c</sup>	Argic Dystric Stagnosol
12	Mullerthal	Loamy	Dolomitic lime <sup>d</sup>	Leptic Dolomitic Dystric Regosol (Loamic)

<sup>a</sup> Sandstone of plots 1–3 belongs to the formation of Luxembourg, whereas that of plots 4–6 is from the Arlon formation.

<sup>b</sup> Sandstone of the Luxembourg formation in plots 7 and 8, but Gilsdorfer sandstone in plot 9.

<sup>c</sup> Marls of the Keuper formation.

<sup>d</sup> Dolomitic lime of the Muschelkalk formation.

microbial community along this gradient for stands under spruce, beech and hornbeam (Kooijman and Smit, 2009). Parent material ranged from sandstone (formation of Luxembourg: Lias) to decalcified marls (Steinmergelkeuper formation: Keuper) and dolomitic limestone (Muschelkalk formation) (Service Géologique, 1998). In both Gaume and Mullerthal, occasional harvesting of trees is the only form of forest management. All selected plots are well-drained.

We selected adjacent deciduous and coniferous plots (20 × 20 m; within 20 m of each other) on the same parent material in both study regions. In the Gaume, we selected six twin plots from an existing network of 43 plots (Verstraeten et al., 2013): three on the Luxembourg formation and three on the Arlon formation (Table 1). Twin plots were grouped together based on those different parent materials, forming a ‘sandy’ and a ‘loamy’ substrate group (Table 1). In the Mullerthal, two twin plots were located on Luxembourg sandstone, one on Gilsdorfer sandstone, one on Keuper marls, one on dolomitic limestone and one on Keuper marls covered by loess: the first three forming the ‘sandy’ substrate group and the other three forming the ‘loamy’ substrate group (Table 1). The twin plot setup of paired adjacent stands and grouping in sandy and loamy substrate groups enabled differentiation between litter input type and parent material effects on SOM quantity and composition. Nevertheless, the difference between both substrate groups is more pronounced in the Mullerthal, due to a larger influence of marls and limestone.

Soils were described and classified following FAO guidelines (Table 1). Mineral soil samples were taken in duplicate with Kopecky rings (100 cm<sup>3</sup>; ø 53 × 50 mm) at four depths (0–5, 5–10, 10–15 and 15–20 cm). Bulk samples were taken from one large soil pit for each plot. Pre-treatment consisted of drying at 40 °C, sieving over 2 mm and milling the <2 mm fraction with a Fritsch pulverisette (400 rpm, 6 min). Leaf and (middle and end) root material was collected from dominant tree species, dried at 40 °C, shredded and milled. Soil material was removed from the roots after drying without using water.

## 2.2. Basic soil properties and carbon stocks

We dried subsamples of milled soil material for one night at 105 °C before we measured total nitrogen and carbon twice with dry combustion on a VarioEL elemental analyser (Elementar). Total carbon equalled organic carbon except for the dolomitic limestone twin plot, for which inorganic carbon was determined with a Sol-TIC (Elementar) extension connected to the elemental analyser. Organic carbon and total nitrogen were used to calculate the C/N

ratio. The pH was determined from a solution with a 1:2.5 wt-to-volume soil/water ratio. SOC stocks (0–20 cm: in tonnes per hectare) were calculated using Eq. (1):

$$\begin{aligned} \text{SOC stock} & \left[ \text{tonnes/hectare or g/dm}^2 \right] \\ & = \text{TOC [g/kg]} \times \text{bulk density} \left[ \text{g/cm}^3 \text{ or kg/dm}^3 \right] \\ & \quad \times \text{depth of layer [dm]} \end{aligned} \quad (1)$$

## 2.3. Thermally assisted hydrolysis and methylation (THM)

Milled soil material was analysed with THM using tetramethylammonium hydroxide (TMAH) (Nierop et al., 2005). We also analysed leaf and root material of all dominant tree species, because different tree species can have different amounts of cutin and suberin. THM has been shown to be accurate in detecting relative differences in lignin and cutin/suberin compounds (Nierop and Verstraeten, 2004). Merits of THM as compared to conventional pyrolysis and extraction were described by Nierop (2001), Nierop et al. (2005). To normalize C content between samples for THM, 5–50 mg (depending on TOC content) of milled soil, leaf or root material was used for THM. The milled material was then mixed with 20 µL of a TMAH solution (25% in water) to split all hydrolysable bonds and transform the resulting hydroxyl and carboxyl groups into their corresponding methyl ethers and methyl esters (Asperger et al., 2001). We added 6 µL of an androstane solution (0.1 µg/µL in cyclohexane) as internal standard. The mixture was pressed on Curie-point wires and shortly dried to vaporize all water. The wires, placed in glass capillaries, were applied manually to the pyrolysis-GC-MS system. Samples were pyrolysed using a Curie-point pyrolyser (Horizon Instruments; 600 °C; 5 sec), connected to a ThermoQuest Trace GC (Waltham, USA) with a fused silica column ZB1-MS (Phenomenex: 30 m × 0.25 mm i.d.) coated with dimethyl poly-cyloxane (film thickness 0.50 µm). Helium was used as carrier gas. Initial temperature was 40 °C (hold time: 1 min, heating rate: 7.0 °C/min). Final temperature was 320 °C (hold time: 10 min). The GC was coupled to a Finnigan Trace MS (Waltham, USA) (*m/z*: 47–500, cycle time: 0.45 sec, ionization energy: 70 eV).

We analysed the data with the Xcalibur software package (Finnigan, Waltham, USA) using a signal-to-noise (S:N) ratio of 3 as the limit of detection and a S:N ratio of 10 as the limit of quantification (Shrivastava and Gupta, 2011). We identified peaks based on retention times and mass spectra using the NIST library (Gaithersburg, USA), ChemSpider (Royal Society of Chemistry, London, UK) and

earlier publications (del Rio et al., 1998; Poerschmann et al., 2005) and quantified them using the internal standard.

#### 2.4. Lignin yield and ratios

Total lignin yield  $\Lambda_{SG}$  (mg/100 mgOC) was calculated by summing up the amounts of the individual lignin monomers (Supplementary Table S1). It is important to mention that only *threo/erythro*-1-(3,4-dimethoxyphenyl)-1,2,3-trimethoxypropane (G14 + G15) and *threo/erythro*-1-(3,4,5-trimethoxyphenyl)-1,2,3-trimethoxypropane (S14 + S15) are monomers of true lignin and are not thought to be derived from other biopolymers while all other monomers can have another origin as well (Nierop et al., 2005; Nierop and Filley, 2007). The syringyl/guaiacyl (S/G) ratio (Table 2), the relative concentration of syringyl and guaiacyl derivatives, is a common lignin proxy for the relative input of angiosperm (deciduous) and gymnosperm (coniferous) woody tissues (Nierop and Filley, 2007). All identified syringyl and guaiacyl derivatives (Supplementary Table S1) were used in the calculation of the S/G ratio except for G18 and the acids (G6, G24, G12; Supplementary Table S1), because those could in the case of deciduous trees also be derived from other biopolymers instead of lignin (Nierop and Filley, 2007). Labda ( $\Gamma$ ) (Table 2), another lignin proxy, calculated as  $G6/(G14 + G15)$  or  $S6/(S14 + S15)$ , indicates side chain shortening, indicative of more decomposed lignin (Nierop and Filley, 2007).

#### 2.5. Cutin and suberin yield and lignin/aliphatic ratio

$C_{16}$  and  $C_{18}$  hydroxy acids can be found in both cutin and suberin. We therefore analysed fresh leaf and root samples of the dominant tree species to select the compounds to be included in the calculation of cutin and suberin yield for coniferous and deciduous forest (Table 2). For coniferous plots, cutin yield was determined by combining the amounts of  $\alpha,\omega$ -dihydroxy  $C_{16}$  fatty acid and 9,10, $\omega$ -trihydroxy  $C_{18}$  fatty acid, whereas for suberin the amounts of the  $\omega$ -hydroxy acids ( $C_{20}$ ,  $C_{24}$  and  $C_{26}$ ) and dicarboxylic acids ( $C_{20}$  and  $C_{22}$ ) were summed (Table 2; Supplementary Table S2). For the deciduous plots  $\alpha,\omega$ -dihydroxy  $C_{16}$  fatty acid represented the cutin yield, whereas 9,10, $\omega$ -trihydroxy  $C_{18}$  fatty acid was taken for suberin (Table 2). The forest type specific calculation of cutin and suberin yield prevents comparison between the two forest types. We used a lignin/aliphatic ratio to analyse if lignin degradation is preferred over aliphatic degradation or vice versa, which seems to be a crucial tipping point in temperate forest SOM composition (Vancampenhout et al., 2010). The lignin/aliphatic ratio

was calculated by dividing the lignin yield with the summed suberin and cutin yield (aliphatic compounds).

#### 2.6. Statistical analysis

We used multi-factor ANOVA (Matlab 7.8.0, R2009a) (The MathWorks, 2009) to check for significant differences in the variables with study area (Gaume or Mullerthal), forest type (deciduous or spruce), substrate (sandy or loamy substrate group) and depth (4 depths) as explaining factors. This was done for the combined data set to check for significant differences between the two study areas, but also for the separate data sets, in the latter case without study area as an explaining factor. We also checked for the occurrence of interaction effects between the different factors. T-tests were used to check for significant differences in the vegetation sample composition. Lilliefors tests were used to check for normality.

### 3. Results

#### 3.1. pH and C/N ratio

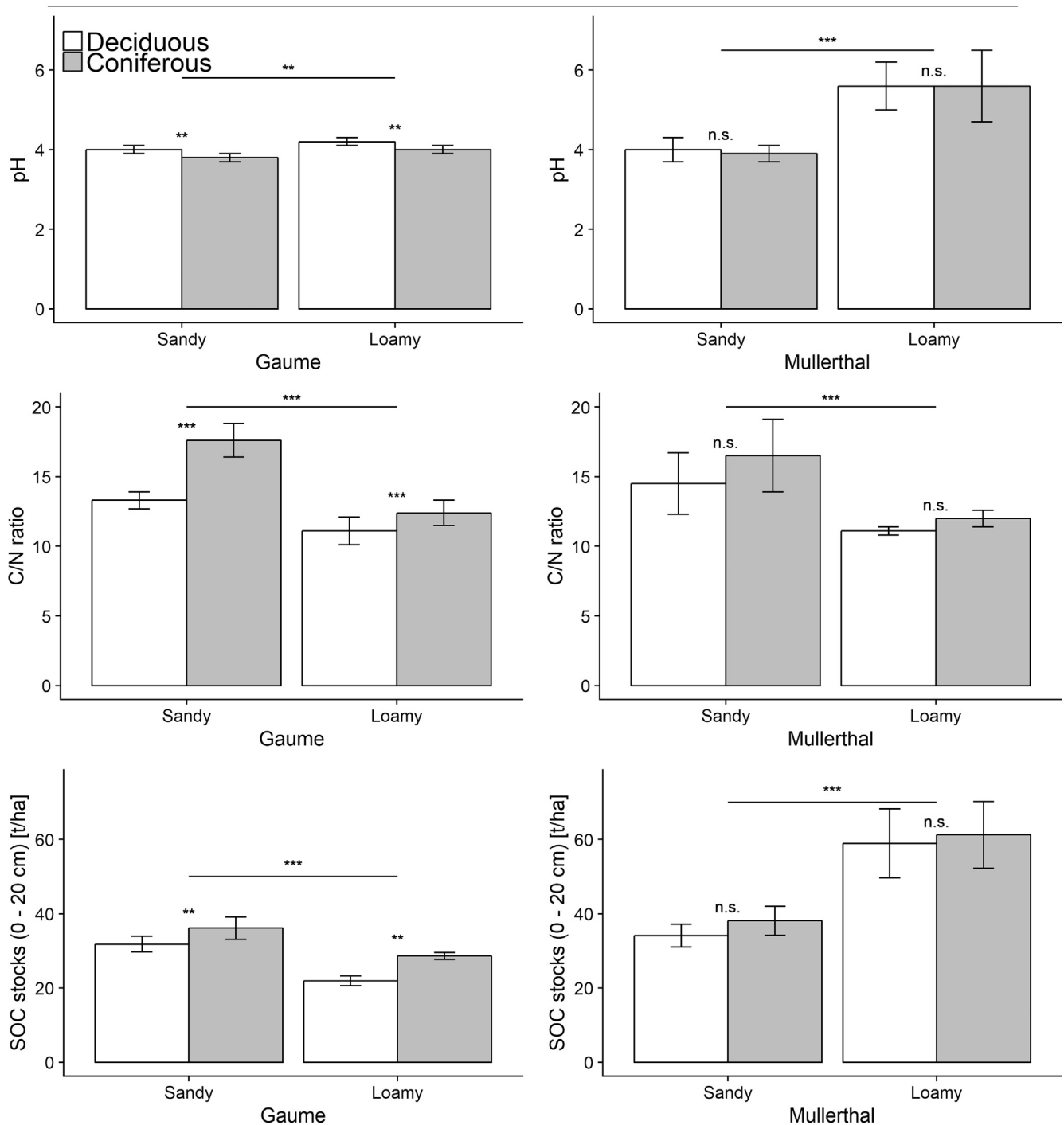
We found a significant ( $P < 0.001$ ) difference in pH values between the Gaume and the Mullerthal (Supplementary Table S3; Fig. 1). However, there was a significant ( $P < 0.001$ ) interaction effect between study area and substrate, with a lower pH for loamy plots in the Gaume than in the Mullerthal (Fig. 1). For the Gaume separately, forest type had a significant effect on pH ( $P < 0.01$ ), with lower values for spruce than for deciduous forest (Fig. 1). Furthermore, lower ( $P < 0.01$ ) pH values were found for sandy than for loamy plots. We found no significant differences in pH between spruce and deciduous forest in the Mullerthal (Fig. 1). However, significant ( $P < 0.001$ ) differences in pH were found between the different substrates in the Mullerthal, with higher pH values ( $P < 0.001$ ) for the loamy plots (Fig. 1).

The C/N ratio did not differ significantly between the two study areas (Supplementary Table S3; Fig. 1). In the Gaume, deciduous plots had significantly ( $P < 0.001$ ) lower C/N ratios compared to the spruce plots (Fig. 1). Moreover, soil C/N ratios were 1.7–5.6 higher for the plots on sandy than on loamy substrate ( $P < 0.001$ ). However, there was a significant ( $P < 0.05$ ) interaction effect between forest type and substrate with larger differences in C/N ratio between forest types in sandy than in loamy plots (Fig. 1). In the Mullerthal, C/N ratios did not differ significantly

**Table 2**  
Lignin and cutin–suberin proxy calculations.

Proxy	Calculation	Proxy for	Source
<i>Lignin ratios</i>			
S/G (Syringyl/Guaiacyl)	S/G (all except G18 and acids)	Relative input of angiosperm and gymnosperm woody tissues	e.g., Nierop and Filley (2007)
$\Gamma_{S \text{ or } G}$	$G6/(G14 + G15)$ or $S6/(S14 + S15)$	Decomposition state; indicator of side shortening	e.g., Nierop and Filley (2007)
<i>Cutin yield</i>			
Deciduous forest	$\alpha,\omega$ -dihydroxy $C_{16}$ fatty acid	Relative input of above ground deciduous litter input	Based on vegetation data
Coniferous forest	$\alpha,\omega$ -dihydroxy $C_{16}$ fatty acid + 9,10, $\omega$ -trihydroxy $C_{18}$ fatty acid	Relative input of above ground coniferous litter input	Based on vegetation data
<i>Suberin yield</i>			
Deciduous forest	9,10, $\omega$ -trihydroxy $C_{18}$ fatty acid	Relative input of below ground deciduous litter input	Based on vegetation data
Coniferous forest	$\omega$ -hydroxy fatty acids ( $C_{20}$ , $C_{24}$ and $C_{26}$ ) + dicarboxylic acids ( $C_{20}$ and $C_{22}$ )	Relative input of below ground coniferous litter input	Based on vegetation data

## pH, C/N ratio and SOC stocks



**Fig. 1.** Substrate and forest type differences in pH, C/N ratio and SOC stocks for the Gaume and the Mullerthal. Values are three plot averages of the top 0–20 cm mineral soil. Error bars are standard errors of the mean. Significance levels used: \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 and n.s. = not significant.

between the two forest types, however C/N ratios were higher for sandy than for loamy plots (P < 0.001) (Fig. 1).

### 3.2. SOC stocks

SOC stocks (0–20 cm) ranged from 19 to 43 t/ha in the Gaume and from 28 to 76 t/ha in the Mullerthal (Fig. 1). Although the difference between the two study areas was significant (P < 0.001) (Supplementary Table S3), there was significant interaction with

substrate (P < 0.001). In the Gaume, SOC stocks were 1.1–3.2 t/ha lower (P < 0.01) for the deciduous plots than for the spruce plots (Fig. 1). Furthermore, in the Gaume, sandy plots had 0.4–4.3 t/ha higher SOC stocks than loamy plots (P < 0.001). In the Mullerthal, the difference in SOC stocks between the two forest types was not significant (Fig. 1). SOC stocks were 3.5–9.0 t/ha higher (P < 0.001) for loamy plots than sandy plots, which is opposite from what was observed in the Gaume (Fig. 1). In summary, SOC stocks differed more between substrates than between forest types and



**Table 3**  
Lignin and cutin/suberin yields and ratios for 11 vegetation samples.

Vegetation sample	$\Lambda_{SG}$ (mg/100 mg veg. mat.)	S/G (ratio)	$\Gamma_G$ (ratio)	Cutin (mg/100 mg veg. mat. <sup>a</sup> )	Suberin (mg/100 mg veg. mat.)
hornbeam – leaves (G) <sup>b</sup>	0.09	1.21	3.40	0.01	<0.01
hornbeam – roots (G)	0.25	0.22	2.22	–	0.01
beech – leaves (G)	0.04	0.95	–	<0.01	–
beech – roots (G)	0.22	0.27	1.71	–	0.03
beech – roots (M) <sup>b</sup>	0.04	0.13	3.09	–	0.01
sycamore – leaves (G)	0.05	0.29	0.81	0.03	<0.01
sycamore – roots (G)	0.07	0.42	4.96	–	<0.01
birch – roots (G)	0.23	1.12	2.15	<0.01	0.01
spruce – needles (G)	0.23	0.14	17.01	0.02	<0.01
spruce – roots (G)	0.17	0.01	4.22	<0.01	0.01
spruce – roots (M)	0.10	0.04	5.05	<0.01	0.01

<sup>a</sup> veg. mat.: vegetation material.

<sup>b</sup> (G) = Gaume, (M) = Mullerthal.

differences between sandy and loamy substrate were opposite for the two study areas.

### 3.3. Lignin: plant material

Lignin yield ( $\Lambda_{SG}$ ) ranged from 0.04 to 0.25 mg/100 mg plant material in 11 fresh leaf and root samples of the dominant tree species (Table 3). Although lignin yield was on average higher for deciduous roots than for deciduous leaves, this difference was not significant (Supplementary Table S4). Lignin S/G ratios of leaves and roots ranged from 0.01 for spruce roots to 1.21 for hornbeam leaves (Table 3). S/G ratios were significantly ( $P < 0.05$ ) lower for spruce needles and roots than for deciduous leaves and roots (Supplementary Table S4). The  $\Gamma_G$ , Labda, ratio ranged from 0.81 for sycamore leaves to 17.01 for spruce needles (Table 3). The high value for spruce needles was confirmed by additional, independent measurements in the laboratory of the University of Utrecht.

### 3.4. Lignin: soil material

Lignin yield was significantly ( $P < 0.001$ ) lower in the Gaume than in the Mullerthal (Supplementary Table S3; Fig. 2). In the Gaume, lignin yield was lower for deciduous forest than for spruce forest ( $P < 0.01$ ). Moreover, lignin yield was lower for loamy plots than for sandy plots ( $P < 0.01$ ). Forest type and substrate had no significant effect on lignin yield in the Mullerthal (Fig. 2). Forest type differences in lignin yield were larger for sandy than for the loamy substrate in both study areas (Fig. 2). Lignin S/G ratios were higher ( $P < 0.05$ ) in the Mullerthal than in the Gaume (Supplementary Table S3; Fig. 2). In the Gaume, S/G ratios were higher ( $P < 0.001$ ) for deciduous plots than for spruce plots (Fig. 2). S/G ratios were not significantly different for sandy and loamy Gaume plots (Fig. 2). In the Mullerthal, forest type was identified as the only significant ( $P < 0.001$ ) factor for the S/G ratio, with higher values for deciduous compared to spruce forest (Fig. 2).

The acid-type lignin compounds (G6, G24, G12, G18; Supplementary Table S1) were present in much higher quantities than 1,3,5-trimethoxybenzene, 2-methyl-1,3,5-trimethoxybenzene and 2-ethyl-1,3,5-trimethoxybenzene, which can be products of tannin. Therefore, we could use the  $\Gamma_G$  (Labda) ratio as an indicator of lignin degradation.  $\Gamma_G$  ratios were higher ( $P < 0.001$ ) in the Gaume than in the Mullerthal (Supplementary Table S3; Fig. 2). However, there was significant interaction between study area and forest type ( $P < 0.05$ ), with a larger difference between the two study areas for the spruce plots (Fig. 2). In the Gaume, there were only significant differences for  $\Gamma_G$  ratio between the two forest types ( $P < 0.05$ ), with  $\Gamma_G$  values, on average, 6.5–14.3 higher for spruce than for deciduous forest (Fig. 2). In the Mullerthal, sub-

strate was the only significant factor ( $P < 0.01$ ).  $\Gamma_G$  ratio values were higher for sandy than for loamy plots in the Mullerthal (Fig. 2).

### 3.5. Cutin and suberin: plant and soil material

There was no significant difference in cutin yield between the two study areas (Supplementary Table S3; Fig. 3). In the Mullerthal, cutin yield was 0.10–7.37 mg/100 mg OC higher for the sandy than the loamy plots ( $P < 0.05$ ). The difference in suberin yield between both study areas was significant ( $P < 0.05$ ) (Supplementary Table S3; Fig. 3), but there was significant interaction with substrate. Suberin yield was higher in the Mullerthal sandy plots than in the Gaume sandy plots, whereas for the loamy plots suberin yield was similar in the two study areas (Fig. 3). In the Mullerthal, suberin yield was 0.14–9.55 mg/100 mg OC higher ( $P < 0.001$ ) for the sandy plots than for the loamy plots (Fig. 3). Cutin and suberin yield ranged from 0 to 0.03 mg/100 mg vegetation material in 11 fresh leaf and root samples of the dominant tree species from the two study areas (Table 3).

The lignin/aliphatic ratio was on average 0.50–4.89 higher ( $P < 0.05$ ) in the Mullerthal than in the Gaume (Supplementary Table S3; Fig. 3). This indicates a higher lignin yield relative to combined cutin and suberin yield in the Mullerthal compared to the Gaume. However, there was significant interaction between study area and substrate ( $P < 0.001$ ). The difference between the loamy plots was much larger than between the sandy plots for the two study areas (Fig. 3). In both the Gaume and the Mullerthal, substrate and depth yielded no significant differences (Fig. 3).

## 4. Discussion

### 4.1. Edaphic and conversion effects on soil geochemistry and SOC stocks

The pH was lower in soils on the same parent material beneath coniferous forest than beneath deciduous forest in the Gaume (Fig. 1). This is in line with earlier findings by Reich et al. (2005), who investigated 14 tree species including the dominant tree species of the present study and found that the effect of a tree species on the soil pH depends on the amount of calcium in the foliage. The absence of a significant effect of forest type on soil pH in the Mullerthal might be explained by the occurrence of hornbeam as dominant species in the deciduous forest plots because hornbeam foliage has a lower calcium content compared to spruce (Reich et al., 2005). Alternatively, a higher buffer capacity of soils on marl and limestone may dampen tree species effects (Vitousek and

## Lignin

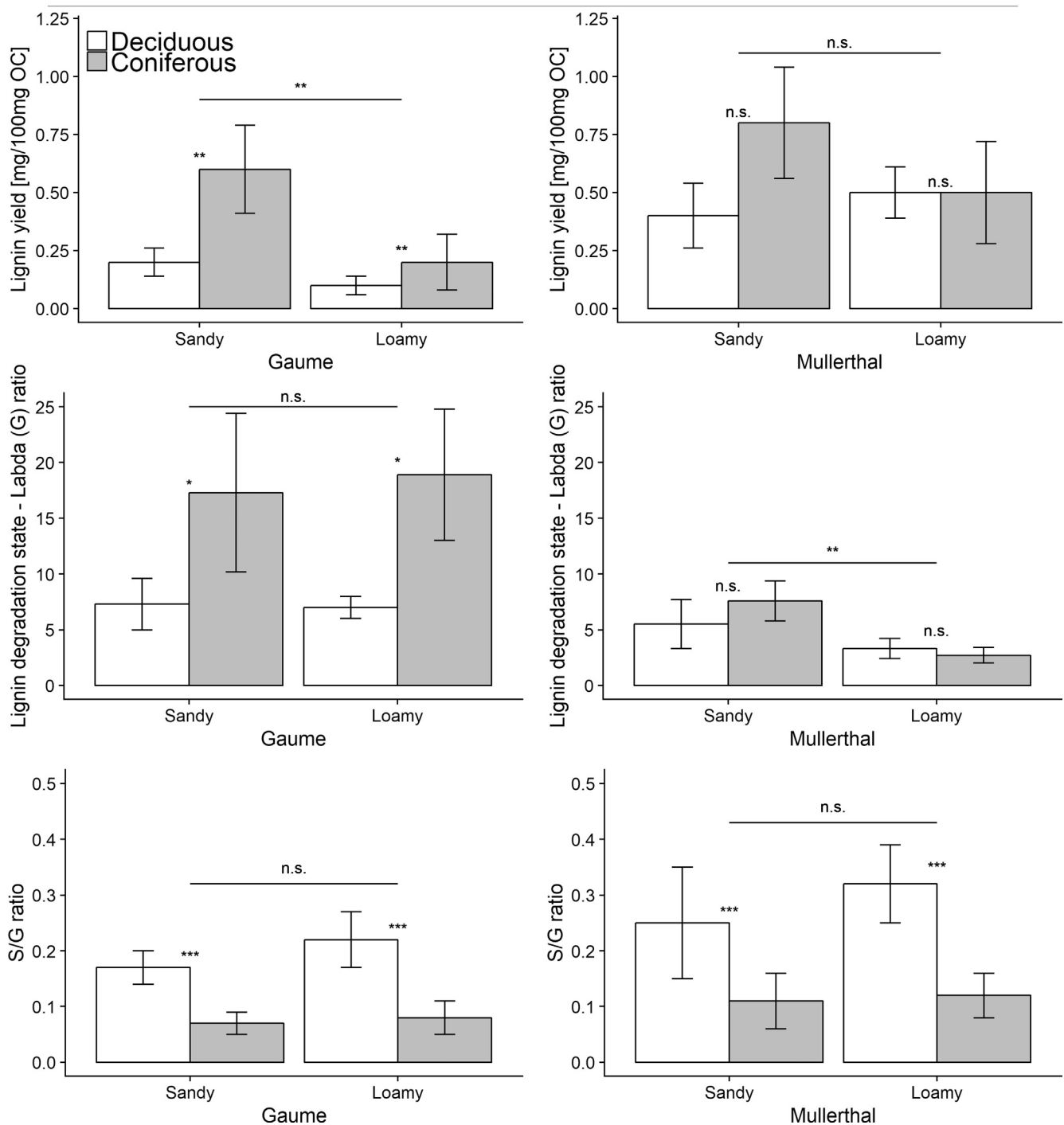
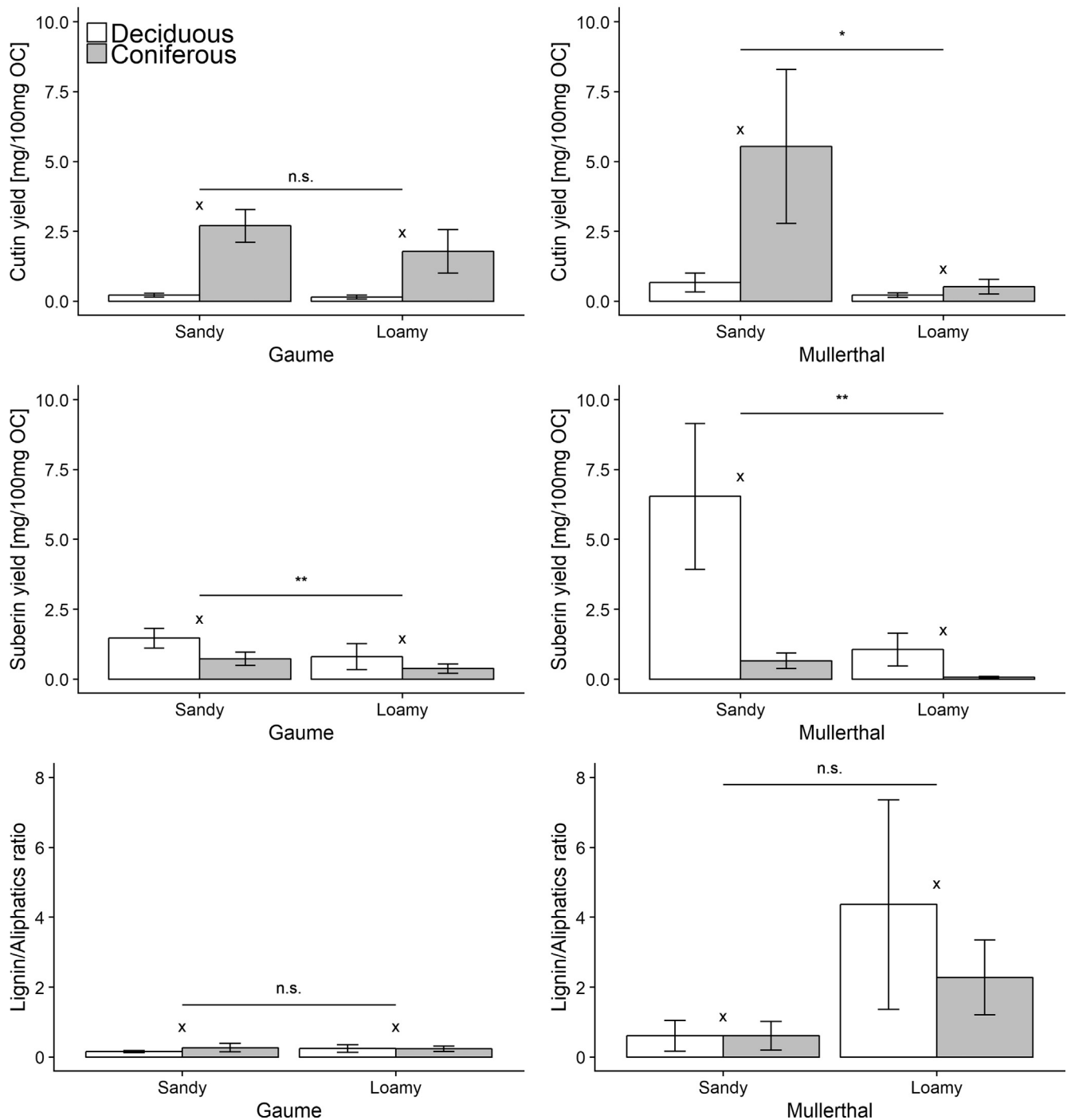


Fig. 2. Substrate and forest type differences in lignin parameters for the Gaume and the Mullerthal. Values are three plot averages of the top 0–20 cm mineral soil. Error bars are standard errors of the mean. Significance levels used: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  and n.s. = not significant.

Chadwick, 2013). Furthermore, pH was lower in the sandy plots than in the loamy plots in both study areas (Fig. 1). The range in pH values was much smaller in the Gaume than in the Mullerthal, due to the more subtle differences in parent material (Table 1). Nevertheless, the pH range in the Gaume spans a typical threshold in soil buffering mechanisms (Vitousek and Chadwick, 2013). This could explain why substrate was found as significant factor for much of the studied parameters in the Gaume as in the Mullerthal.

SOC stocks (0–20 cm), ranging from 19 to 43 t/ha in the Gaume and from 28 to 76 t/ha in the Mullerthal (Fig. 1), are significantly higher in the higher pH ranges and are relatively low in comparison with some earlier studies (Post et al., 1982; Lettens et al., 2005). Post et al. (1982) estimated carbon densities in temperate European forest soils to be 90 t/ha on average. Lettens et al. (2005), reported average SOC stocks (0–20 cm) of 66 t/ha for broadleaved forests and 71 t/ha for coniferous forests in Belgium.

## Cutin and Suberin



**Fig. 3.** Substrate and forest type differences in cutin/suberin parameters for the Gaume and the Mullerthal. Values are three plot averages of the top 0–20 cm mineral soil. Error bars are standard errors of the mean. Significance levels used: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  and n.s. = not significant. No values (x) are shown for forest type because of the different calculation of both cutin and suberin yield for deciduous and coniferous forest (See Table 2).

The larger differences in SOC stocks between different substrates than between forest types (Fig. 1), indicate that parent material has a larger effect on carbon sequestration than forest type in this study. The trend in the Mullerthal is expected, because with loamy substrate more carbon can be physically or chemically protected by interaction with clay particles (Hagedorn et al., 2001). These findings are in line with a recent study that identified the fine min-

eral fraction as the key driver of SOC stabilization and storage in most soils, with vegetation characteristics as an additional factor that further refined SOC quantification (Wiesmeier et al., 2018). Moreover, in restored grasslands even relatively small differences in edaphic properties such as exchangeable soil calcium and clay content were shown to have large effects on SOC stocks when integrated on a longer time scale (O'Brien et al., 2015). Our results sug-



gest that this may also apply to forests, as we observed differences in SOC stocks and other edaphic parameters in the Gaume despite relatively minor textural differences.

#### 4.2. Edaphic and tree species effects on SOM lignin compounds

The use of twin plots with deciduous and coniferous forest plots on the same parent material enabled us to evaluate the relative importance of litter input chemistry and substrate-related edaphic factors such as pH on the molecular SOM composition and SOC stocks in forest soils. Hence, it contributes to the ongoing debate on the relative importance of litter composition versus soil environment as determining factors for SOM persistence (Schmidt et al., 2011; Lehmann and Kleber, 2015). Lignin yield strongly depends on litter input, but differences in lignin yields upon conversion are more pronounced in acid soils compared to soils with a more favourable pH (Fig. 2). Moreover, tree species effects on lignin degradation seem strongly site dependent, with considerably larger differences upon conversion in the Gaume plots (Fig. 2).

The  $\Gamma_G$  ratio was higher under coniferous forest than under deciduous forest, particularly in the Gaume (Fig. 2). The more degraded state of lignin could be explained by increased fungal activity under long-term acidic conditions (Camarero et al., 1994; Nierop and Verstraten, 2003), as the lower pH values under coniferous forest in the Gaume (Fig. 1) favour the growth of lignin-decomposing fungi (Rousk et al., 2009; Floudas et al., 2012). This could explain why lignin was, according to the  $\Gamma_G$  ratio, less degraded in the loamy Mullerthal plots (Fig. 2) where pH ranged from 5.8 to 7.1. In addition to pH, the higher C/N ratio observed in the more acidic soils beneath spruce forest (Fig. 1) could also explain the more degraded lignin in the coniferous plots: earlier studies (Reid, 1983; Rinkes et al., 2016) showed that lignin degradation is inhibited by higher amounts of nitrogen relative to carbon. These effects of pH and relative nitrogen content however do not explain the remarkable difference in lignin decomposition between the sites. One explanation may be that the Gaume is an old-growth forest that has been continuously forested at least since 1775 (Ferraris, 1777), whereas two of the Mullerthal plots were probably reforested in 1850 (Kooijman, 2010). Earlier work (Morriën et al., 2017) showed that past land uses can have important legacy effects on the effectivity of lignin degradation. Finally, a higher bioturbation due to a higher earthworm presence at neutral to alkaline pH values (Reich et al., 2005) may also affect lignin degradation.

The significantly higher S/G ratios in deciduous forest soils compared to those observed in coniferous forest soils (Fig. 2) confirm earlier findings (Hedges and Mann, 1979; Vancampenhout et al., 2010) that vegetation type, through litter input, is an important factor determining the extractable SOM composition in maritime temperate forests when climate conditions are similar: the higher S/G values were found in plots with deciduous litter input and S/G values near zero were found in plots with coniferous litter input (Fig. 2). Nevertheless, our analysis of different litter types not only confirms the differences between angiosperms and gymnosperms regarding S/G ratio, but also shows that there can be variations according to species and plant organs (Table 3). In almost all cases, we did not find a legacy effect of deciduous vegetation in the lignin composition in the spruce plots. This can be explained by the earlier discussed higher lignin decomposition under most spruce plots (Fig. 2).

In summary, lignin yield, composition and degradation state were influenced both by litter input chemistry as well as by edaphic (soil-related) factors. Moreover, there appear to be important interaction effects between the two, as the relative importance of parent material and litter quality was site-specific. Previous research also revealed complex interconnections between climate, C inputs, C pools and soil properties in regulating SOC dynamics

(Luo et al., 2017). Luo et al. (2017) showed that soil properties accounted for 24% of the relative influence on the SOC change rate and thus this cannot be ignored when modelling SOC dynamics. Related to this, Angst et al. (2018) demonstrated the necessity to consider parent material differences when estimating and predicting SOC stocks. This is in line with the relative importance of substrate observed in our study and highlights that substrate should always be taken into account in forest management.

#### 4.3. Cutin and suberin: leaf litter vs root input

We studied cutin and suberin to assess the relative importance of root and litter input on the SOM composition in converted and non-converted forests. High cutin yields in spruce plots (Fig. 3) are caused by a high cutin content of coniferous needles compared to deciduous leaves (Table 3). However, similar to our observations for lignin, these tree type effects are absent from the Mullerthal loamy plots (Fig. 3). For suberin yield, the picture is less clear. Site seems to be more important determinant in this case, with larger differences between the forest types in the Mullerthal than in the Gaume (Fig. 3). This may be a species effect, as beech roots had a higher suberin content than hornbeam (Table 3) and beech is the dominant species in the sandy Mullerthal plots compared to hornbeam in most Gaume plots. Lastly, for the lignin/aliphatic ratio again the loamy Mullerthal plots stood out and had more lignin relative to aliphatics especially under deciduous trees (Fig. 3).

## 5. Conclusions

SOC stocks in the Gaume and Mullerthal regions were influenced more by parent material than by forest type. Moreover, although lignin yield strongly depends on litter input, differences in lignin yield upon conversion were more pronounced in acid soils compared to soils with a more favourable pH. Tree species effects on lignin degradation seem to be strongly site dependent, with considerably larger differences upon conversion in the Gaume plots. Higher lignin decomposition under spruce removed traces of former deciduous input from the lignin composition in converted forest plots. In summary, lignin yield, composition and degradation state were influenced both by litter input chemistry as by the edaphic (soil-related) context. Moreover, there appear to be important interaction effects between the two, as the relative importance of parent material and litter quality was site-specific. These conclusions seem also to be valid for cutin yield, as tree type effects were absent from the Mullerthal loamy plots with more favourable pH values. We suggest that models used to estimate carbon stocks should always combine data on vegetation history and edaphic context. For forest management this study shows that if forest conversion is applied to increase forest SOM stocks, as climate change mitigation measure, this can only be a success when substrate differences are taken into account, including relatively minor substrate-directed differences in soil properties such as pH and texture.

#### Declaration of Competing Interest

None.

#### Acknowledgements

This study was co-funded by UvA, The Netherlands, and K.U. Leuven, Belgium. We thank the Cantonnement de Virton (Forestry in the Gaume) and the local Luxembourg authorities for field work permission. We thank Joke Westerveld, Leo Hoitinga, Chiara Cerli and Jorien Schoorl for their kind support with laboratory work at the University of Amsterdam. We also thank Michaël van Raem-

donck and Audrey Miserez (both KU Leuven) for their help during both field work periods. Lastly, we express our gratitude to two anonymous referees and the editors J. Volkman and I. Kögel-Knabner for their careful reviews.

## Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.orggeochem.2019.05.006>.

Associate Editor—Ingrid Kögel-Knabner

## References

- Angst, G., Messinger, J., Greiner, M., Häusler, W., Hertel, D., Kirfel, K., Kögel-Knabner, I., Leuschner, C., Rethemeyer, J., Mueller, C.W., 2018. Soil organic carbon stocks in topsoil and subsoil controlled by parent material, carbon input in the rhizosphere, and microbial-derived compounds. *Soil Biology and Biochemistry* 122, 19–30.
- Asperger, A., Engewald, W., Fabian, G., 2001. Thermally assisted hydrolysis and methylation – a simple and rapid online derivatization method for the gas chromatographic analysis of natural waxes. *Journal of Analytical and Applied Pyrolysis* 61, 91–109.
- Batjes, N.H., 2014. Total nitrogen and carbon in the soils of the world. *European Journal of Soil Science* 65, 10–21.
- Bouezmarni, M., Denne, P., Debbaut, V., 2009. Carte hydrogéologique de Wallonie; Meix-Devant-Virton – Virton.
- Camarero, S., Galletti, G.C., Martínez, A.T., 1994. Preferential degradation of phenolic lignin units by two white rot fungi. *Applied and Environmental Microbiology* 60, 4509–4516.
- Cremer, M., Kern, N.V., Prietzel, J., 2016. Soil organic carbon and nitrogen stocks under pure and mixed stands of European beech, Douglas fir and Norway spruce. *Forest Ecology and Management* 367, 30–40.
- D'Amato, A.W., Bradford, J.B., Fraver, S., Palik, B.J., 2011. Forest management for mitigation and adaptation to climate change: insights from long-term silviculture experiments. *Forest Ecology and Management* 262, 803–816.
- del Rio, J.C., McKinney, D.E., Knicker, H., Nanny, M.A., Minard, R.D., Hatcher, P.G., 1998. Structural characterization of bio- and geo-macromolecules by off-line thermochemolysis with tetramethylammonium hydroxide. *Journal of Chromatography A* 823, 433–448.
- Ferraris, le C. de, 1777. Carte de Cabinet des Pays-Bas Autrichiens, Reissud i. ed. *Bibliothèque Royale de Belgique, Bruxelles* BE.
- Floudas, D., Binder, M., Riley, R., Barry, K., Blanchette, R.A., Henrissat, B., Martínez, A. T., Otilar, R., Spatafora, J.W., Yadav, J.S., Coutinho, P.M., Vries, R.P. De, Ferreira, P., Findley, K., Foster, B., 2012. The paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. *Science* 336, 1715–1719.
- Gleixner, G., 2013. Soil organic matter dynamics: a biological perspective derived from the use of compound-specific isotopes studies. *Ecological Research* 28, 683–695.
- Hagedorn, F., Maurer, S., Egli, P., Blaser, P., Bucher, J.B., Siegwolf, R., 2001. Carbon sequestration in forest soils: effects of soil type, atmospheric CO<sub>2</sub> enrichment, and N deposition. *European Journal of Soil Science* 52, 619–628.
- Hedges, J.L., Mann, D.C., 1979. The characterization of plant tissues by their lignin oxidation products. *Geochimica et Cosmochimica Acta* 43, 1803–1807.
- Jandl, R., Lindner, M., Vesterdal, L., Bauwens, B., Baritz, R., Hagedorn, F., Johnson, D. W., Minkinen, K., Byrne, K.A., 2007. How strongly can forest management influence soil carbon sequestration? *Geoderma* 137, 253–268.
- Kirschbaum, M.U.F., 2000. Will changes in soil organic carbon act as a positive or negative feedback on global warming? *Biogeochemistry* 48, 21–51.
- KMI, 2014. Database of the Belgian Royal Meteorological Institute [WWW Document]. URL <<http://www.kmi.be/>> (accessed 10.14.14).
- Kögel-Knabner, I., 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil Biology and Biochemistry* 34, 139–162.
- Kolattukudy, P.E., 1980. Biopolyester membranes of plants: cutin and suberin. *Science* 208, 990–1000.
- Kooijman, A.M., 2010. Litter quality effects of beech and hornbeam on undergrowth species diversity in Luxembourg forests on limestone and decalcified marl. *Journal of Vegetation Science* 21, 248–261.
- Kooijman, A.M., Cammeraat, E., 2010. Biological control of beech and hornbeam affects species richness via changes in the organic layer, pH and soil moisture characteristics. *Functional Ecology* 24, 469–477.
- Kooijman, A.M., Martínez-Hernandez, G.B., 2009. Effects of litter quality and parent material on organic matter characteristics and N-dynamics in Luxembourg beech and hornbeam forests. *Forest Ecology and Management* 257, 1732–1739.
- Kooijman, A.M., Smit, A., 2009. Paradoxical differences in N-dynamics between Luxembourg soils: litter quality or parent material? *European Journal of Forest Research* 128, 555–565.
- Lal, R., 2005. Forest soils and carbon sequestration. *Forest Ecology and Management* 220, 242–258.
- Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. *Nature* 528, 60–68.
- Letten, S., Van Orshoven, J., Van Wesemael, B., De Vos, B., Muys, B., 2005. Stocks and fluxes of soil organic carbon for landscape units in Belgium derived from heterogeneous data sets for 1990 and 2000. *Geoderma* 127, 11–23.
- Luo, Z., Feng, W., Luo, Y., Baldock, J., Wang, E., 2017. Soil organic carbon dynamics jointly controlled by climate, carbon inputs, soil properties and soil carbon fractions. *Global Change Biology* 23, 4430–4439.
- Meteo Luxembourg, 2014. Climatology [WWW Document]. URL <<http://www.meteolux.lu>> (accessed 10.14.14).
- Morriën, E., Hannula, S.E., Snoek, L.B., Helmsing, N.R., Zweers, H., de Hollander, M., Soto, R.L., Bouffaud, M.L., Buée, M., Dimmers, W., Duyts, H., Geisen, S., Giralanda, M., Griffiths, R.I., Jørgensen, H.B., Jensen, J., Plassart, P., Redecker, D., Schmelz, R. M., Schmidt, O., Thomson, B.C., Tisserant, E., Uroz, S., Winding, A., Bailey, M.J., Bonkowski, M., Faber, J.H., Martin, F., Lemanceau, P., de Boer, W., van Veen, J.A., van der Putten, W.H., 2017. Soil networks become more connected and take up more carbon as nature restoration progresses. *Nature Communications*. 8, Article number: 14349.
- Naudts, K., Chen, Y., McGrath, M.J., Ryder, J., Valade, A., Otto, J., Luysaert, S., 2016. Europe's forest management did not mitigate climate warming. *Science* 351, 597–600.
- Nierop, K.G.J., 2001. Temporal and vertical organic matter differentiation along a vegetation succession as revealed by pyrolysis and thermally assisted hydrolysis and methylation. *Journal of Analytical and Applied Pyrolysis* 61, 111–132.
- Nierop, K.G.J., Filley, T.R., 2007. Assessment of lignin and (poly)-phenol transformations in oak (*Quercus robur*) dominated soils by <sup>13</sup>C-TMAH thermochemolysis. *Organic Geochemistry* 38, 551–565.
- Nierop, K.G.J., Filley, T.R., 2008. Simultaneous analysis of tannin and lignin signatures in soils by thermally assisted hydrolysis and methylation using <sup>13</sup>C-labeled TMAH. *Journal of Analytical and Applied Pyrolysis* 83, 227–231.
- Nierop, K.G.J., Verstraten, J.M., 2003. Organic matter formation in sandy subsurface horizons of Dutch coastal dunes in relation to soil acidification. *Organic Geochemistry* 34, 499–513.
- Nierop, K.G.J., Verstraten, J.M., 2004. Rapid molecular assessment of the bioturbation extent in sandy soil horizons under pine using ester-bound lipids by on-line thermally assisted hydrolysis and methylation-gas chromatography/mass spectrometry. *Rapid Communications in Mass Spectrometry* 18, 1081–1088.
- Nierop, K.G.J., Preston, C.M., Kaal, J., 2005. Thermally assisted hydrolysis and methylation of purified tannins from plants. *Analytical Chemistry* 77, 5604–5614.
- O'Brien, S.L., Jastrow, J.D., Grimley, D.A., Gonzalez-Meler, M.A., 2015. Edaphic controls on soil organic carbon stocks in restored grasslands. *Geoderma* 251–252, 117–123.
- Poerschmann, J., Gathmann, A., Augustin, J., Langer, U., Górecki, T., 2005. Molecular composition of leaves and stems of genetically modified Bt and near-isogenic non-Bt maize—Characterization of lignin patterns. *Journal of Environmental Quality* 34, 1508–1518.
- Post, W.M., Emanuel, W.R., Zinke, P.J., Stangenberger, A.G., 1982. Soil carbon pools and world life zones. *Nature* 298, 156–159.
- Reich, P.B., Oleksyn, J., Modrzyński, J., Mrozinski, P., Hobbie, S.E., Eissenstat, D.M., Chorover, J., Chadwick, O.A., Hale, C.M., Tjoelker, M.G., 2005. Linking litter calcium, earthworms and soil properties: a common garden test with 14 tree species. *Ecology Letters* 8, 811–818.
- Reid, I.D., 1983. Effects of nitrogen supplements on degradation of Aspen wood lignin and carbohydrate components by *Phanerochaete chrysosporium*. *Applied and Environmental Microbiology* 45, 830–837.
- Rinkes, Z.L., Bertrand, I., Amin, B.A.Z., Grandy, A.S., Wickings, K., Weintraub, M.N., 2016. Nitrogen alters microbial enzyme dynamics but not lignin chemistry during maize decomposition. *Biogeochemistry* 128, 171–186.
- Rousk, J., Brookes, P.C., Bååth, E., 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Applied and Environmental Microbiology* 75, 1589–1596.
- Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. *Nature* 478, 49–56.
- Service Géologique, 1998. Carte géologique du Grand-Duché de Luxembourg [WWW Document]. URL <<http://www.pch.public.lu/fr/publications/g/carte-geologique-grand-duche-luxembourg/carte-geologique-grand-duche-luxembourg.pdf>> (accessed 12.6.16).
- Shrivastava, A., Gupta, V., 2011. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chronicles of Young Scientists* 2, 21–25.
- Six, J., Jastrow, J.D., 2002. Organic matter turnover. In: Lal, R. (Ed.), *Encyclopedia of Soil Science*, Marcel Dekker, New York, pp. 936–942.
- Six, J., Paustian, K., 2014. Aggregate-associated soil organic matter as an ecosystem property and a measurement tool. *Soil Biology and Biochemistry* 68, A4–A9.
- Spielvogel, S., Prietzel, J., Kögel-Knabner, I., 2016. Stand scale variability of topsoil organic matter composition in a high-elevation Norway spruce forest ecosystem. *Geoderma* 267, 112–122.
- Stocker, T., Qin, D., Plattner, G., Tignor, M., 2013. IPCC, 2013: climate change 2013: the physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel.
- The MathWorks, 2009. MATLAB version 7.8.0 R2009a.
- Thevenot, M., Dignac, M.F., Rumpel, C., 2010. Fate of lignins in soils: a review. *Soil Biology and Biochemistry* 42, 1200–1211.
- Vancampenhout, K., De Vos, B., Wouters, K., Van Calster, H., Swennen, R., Buurman, P., Deckers, J., 2010. Determinants of soil organic matter chemistry in maritime temperate forest ecosystems. *Soil Biology and Biochemistry* 42, 220–233.

- Verstraeten, G., Baeten, L., Van den Broeck, T., De Frenne, P., Demey, A., Tack, W., Muys, B., Verheyen, K., 2013. Temporal changes in forest plant communities at different site types. *Applied Vegetation Science* 16, 237–247.
- Verstraeten, G., Vancampenhout, K., Desie, E., De Schrijver, A., Hlava, J., Schelfhout, S., Verheyen, K., Muys, B., 2018. Tree species effects are amplified by clay content in acidic soils. *Soil Biology and Biochemistry* 121, 43–49.
- Vitousek, P.M., Chadwick, O.A., 2013. Pedogenic thresholds and soil process domains in basalt-derived soils. *Ecosystems* 16, 1379–1395.
- Wiesmeier, M., Urbanski, L., Hobley, E., Lang, B., von Lütow, M., Marin-Spiotta, E., van Wesemael, B., Rabot, E., Lief, M., Garcia-Franco, N., Wollschläger, U., Vogel, H.-J., Kögel-Knabner, I., 2018. Soil organic carbon storage as a key function of soils – a review of drivers and indicators at various scales. *Geoderma* 333, 149–162.