Local soil characteristics determine the microbial communities under forest understory plants along a latitudinal gradient

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Received 8 July 2018; accepted 4 March 2019
Available online 15 March 2019

Abstract

The soil microbial community is essential for maintaining ecosystem functioning and is intimately linked with the plant community. Yet, little is known on how soil microbial communities in the root zone vary at continental scales within plant species. Here we assess the effects of soil chemistry, large-scale environmental conditions (i.e. temperature, precipitation and nitrogen deposition) and forest land-use history on the soil microbial communities (measured by phospholipid fatty acids) in the root zone of four plant species (Geum urbanum, Milium effusum, Poa nemoralis and Stachys sylvatica) in forests along a 1700 km latitudinal gradient in Europe.

Soil microbial communities differed significantly among plant species, and soil chemistry was the main determinant of the microbial community composition within each plant species. Influential soil chemical variables for microbial communities were plant species-specific; soil acidity, however, was often an important factor. Large-scale environmental conditions, together with soil chemistry, only explained the microbial community composition in M. effusum and P. nemoralis. Forest land-use history did not affect the soil microbial community composition.
Our results underpin the dominant role of soil chemistry in shaping microbial community composition variation within plant species at the continental scale, and provide insights into the composition and functionality of soil microbial communities in forest ecosystems.

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**Keywords:** Ancient forests; Phospholipid fatty acids; Soil chemistry; Soil fungi and bacteria; Temperature and precipitation; Variation partitioning

### Introduction

Understorey plant species in temperate forests play a critical role in maintaining forest ecosystem functioning. Yet, little is known about the soil microbial communities under the understorey plant species (Gilliam 2007). Soil microbial communities such as fungi and bacteria are highly diverse and are essential for key ecosystem functions such as carbon (C) and nutrient cycling (Paul 2014; Wagg, Bender, Widmer, & van der Heijden 2014). The key aspect of elucidating the ecological role of soil microbial communities under plants is to find the driving factor for their compositional differences. Many studies have focused on the soil microbial community composition between different plant species and found that plant species identity is a critical driver for soil microbial community composition mainly through root exudates and litter chemistry (Bakker, Bradeen, & Kinkel 2013; Martinez-Garcia, Richardson, Tylianakis, Peltzer, & Dickie 2015). Within species, populations occurring in different regions experience sometimes very contrasting resources and conditions. These resources and conditions, such as soil chemistry, climate, nitrogen (N) deposition and land-use history, can strongly affect the intraspecific variation in terms of soil microbe communities of the root zone. Yet, knowledge of the importance of abiotic factors in driving soil microbial community composition within plant species is largely lacking.

Among the abiotic environmental factors, soil chemistry can affect soil microbial communities substantially because it relates to many essential resources for soil fungi and bacteria (e.g., the available soil C, N, phosphorus (P) and many other macro- and microelements) (Schmidt et al. 2011; Brockett, Prescott, & Grayston 2012; Paul 2014). Soil nutrient availability (e.g., N and P) and acidity drives the activities of soil fungi and bacteria with respect to nutrient utilization and decomposition rate (Yin, Phillips, Liang, Xu, & Liu 2016). For instance, lower pH generally benefits soil fungi while higher pH facilitates soil bacteria (Stevens et al. 2011). In addition, the composition of microbial communities can shift from fungi-dominated to bacteria-dominated with lower soil C concentrations (Hu et al. 2017). In another study, Richter, Schoning, Kahl, Bauhus, and Rues (2018) examined fungi and bacterial biomass in the mineral soil (0–10 cm) in 150 temperate forests across Germany and found that soil nutrients and pH are strongly correlated with the abundance of Gram-positive bacteria and *Actinobacteria*.

At larger geographical scales, forests along latitudinal gradients vary in terms of their climatic conditions and N deposition loads. Forest soil fungal and bacterial communities may shift corresponding to the variation in temperature, precipitation and atmospheric N deposition (Staddon, Trevors, Duchesne, & Colombo 1998; Dentener et al. 2006; Castro, Classen, Austin, Norby, & Schadt 2010). Staddon et al. (1998) found ambient temperature to be one of the main determinants for the decreasing diversity of the soil microbial community along an 800 km latitudinal gradient in Western Canada. Likewise, different precipitation regimes altered the fungal community composition and the relative abundance of two dominant bacterial phyla (i.e., *Acidobacteria* and *Proteobacteria*) in a deciduous forest ecosystem in Eastern America (Castro et al. 2010). Many non-crop vegetation types in Western and Eastern Europe experience exceedingly high N deposition loads (higher than 10 kg N ha$^{-1}$ year$^{-1}$) (Dentener et al. 2006), and the high N deposition shifts the composition of plant and soil microbial communities (Fierer et al. 2012). Collectively, these large-scale environmental conditions may contribute in explaining soil microbial community composition.

Finally, in Europe and eastern North America, many temperate forests are post-agricultural and bear the imprint of previous land-use. Previous land-use shapes specific soil abiotic and biotic conditions (Jangid et al. 2011), and the legacy of land-use change on plant and soil microbial communities may persist for decades to centuries (Aggemyr & Cousins 2012; Bachelot et al. 2016). Thus, the soil microbial communities of ancient forests (age >200 years according to historical maps) and more recently established forests on former agricultural land (age <200 years) (Hermy & Verheyen 2007) may differ. Surprisingly, despite its ecological importance, the soil microbial community (with a focus on fungi and bacteria) has never been compared between ancient and recent temperate forests at the European scale. Tracking the impact strength of land-use history on soil microbial community can create baselines to refer to and further our understanding on the importance of soil abiotic and biotic conditions with respect to temporal dynamics of soil biota.

Here we focus on the effects of abiotic factors (soil chemistry, large-scale environmental conditions and land-use history) on soil microbial community composition under four understorey plant species, which differ in terms of their colonization rate and life form. Phospholipid fatty acid-based soil
microbial community composition was determined using root zone soils under each plant species, collected along a 1700 km latitudinal gradient in Europe. We hypothesized that (1) soil microbial community composition differs between the four plant species; (2) soil microbial community composition in each plant species is significantly driven by three abiotic factors (soil chemistry, large-scale environmental conditions and land-use history); and (3) higher soil nutrients and pH can benefit or impair specific fungi and bacteria groups, resulting in shifts in soil microbial community composition.

Materials and methods

Study area

We focused on deciduous forests because they are the most abundant forest type in rural landscape across Europe and have a highly diverse understorey species composition. Forest continuity based on existing evidence was used to distinguish ancient and recent forests. Ancient forests were those forested continuously since at least a specified date, but this date varies among regions depending on the availability of historical site information and ranges from 1600 to 1820 (Hermy, Honnay, Firbank, Grashof-Bokdam, & Lawesson 1999). Recent forests were those afforested on previously agricultural lands (Flinn & Vellend 2005) and mostly established in the 19th century except in Poland, where recent forests were established 15–40 years ago. In several regions, only a small fraction of the actual forest cover can be referred to as ‘ancient’. These ancient forests have no historical record (mainly cartographical) of agricultural land-use and have been continuously wooded for at least ca. 150–400 years (Hermy et al. 1999; Flinn and Vellend 2005; Hermy and Verheyen 2007; De Frenne et al. 2011). We selected eight regions based on species occurrences along the latitudinal gradient, i.e., Northern France (NF), Belgium (Be), Poland (Po), Western Germany (WG), Eastern Germany (EG), Southern Sweden (SS), Central Sweden (CS) and Estonia (Es) (Fig. 1). Within each region, two 5 × 5 km landscape windows were selected. Within each window, one ancient and one recent forest was selected to assess the effects of land-use history. The size of the forests ranged from 0.5 ha to 5.4 ha (Valdes et al. 2015).

Soil sampling

In June and July 2015, we selected Geum urbanum L. (Rosaceae), Milium effusum L. (Poaceae), Poa nemoralis L. (Poaceae) and Stachys sylvatica L. (Lamiaceae) across selected forests as study species. The selected species cover two plant life forms, i.e., grasses (M. effusum and P. nemoralis) and forbs (G. urbanum and S. sylvatica), and have different capacities to colonize ecotones of ancient and recent (land-use history) deciduous forests. We searched each forest for patches of four understorey species, and the sampling site for each species within the patch was at least 50 m away from each other. When the four species did not occur in the same forest patch in a certain region, we sampled from other patches but with consistent land-use history within the region. For each sampling site, the focal plant was surrounded by multiple individuals of the same species. Tree species distributed within a radius of 2.5 m to the focal individuals were recorded together with an estimation on canopy cover. See Appendix A: Table S1 in Supplementary material for tree species composition. We selected healthy plant individuals (no damage from herbivores or pathogens) for each understorey species at least 10 m away from the nearest forest edge (4 sites per region per species = 2 windows × 2 forests). We cut the stem of each plant at about 1 cm above the ground. After removing the litter layer around the focal plant individual, 0–10 cm soil samples were taken with augers with a diameter of 3 cm around each plant. Visible plant roots, debris and stones were removed immediately. In total, we had 118 soil samples instead of the expected 128 samples (8 regions × 4 sites × 4 species) because some species were absent from some forests in the region (see Appendix A in Supplementary material: soil sampling for specific absence). Soil samples were sent with ice bags to the central lab in Belgium and were immediately sieved through a 1-mm mesh upon arrival to filter out finer roots and debris. The mesh was cleaned and sterilized with 75% ethanol before processing the next sample. A subsample of each soil sample was taken and dried at 40 °C for 48 h for subsequent chemical analyses. The remaining soils were stored at −18 °C until the start of the extraction of soil microbial biomass.

Soil microbial biomass

Phospholipid fatty acids (PLFAs) were extracted and determined following Huygens et al. (2011). In brief, total lipids were extracted from 6 g freeze-dried soil using phosphate buffer/chloroform/methanol (0.9:1:2) before being loaded on a silica-filled solid-phase extraction column for purification. Neutral- and glycolipids were washed off from the extraction column using chloroform and acetone, respectively, before eluting the phospholipids with methanol. Phospholipids were subsequently transmethylated using methanolic KOH and the prepared fatty acids methyl esters (FAME) were quantified using gas chromatography-mass spectrometry (GC–MS, Trace GC-DSQ, Thermo Fisher, USA) equipped with a VF 23-MS column (60 m, 0.25 mm i.d., 0.25 μm film thickness; Varian, USA). Methylloleic acid (Me18:0) was used as internal standard. The biomass represented by each biomarker was calculated based on the PLFA concentrations (μg/g). In total, 35 PLFA biomarkers were detected. We did not consider three of the biomarkers because of the low frequency in our samples (two biomarkers) and the unrecognizable identity (one biomarker). The retained 32 useful biomarkers accounted for 89–94% of the total biomass.
We classified 17 biomarkers in different functional groups (*Actinobacteria*, non-specific bacteria, Gram-positive bacteria, Gram-negative bacteria and fungi) and the remaining 15 biomarkers as unclassified (see Appendix A: Table S2 in Supplementary material).

**Soil chemistry**

Soils were combusted at 1200 °C, and the gases were measured using a thermal conductivity detector in a CNS elemental analyser (vario Macro Cube, Elementar, Germany) for total carbon (C) and nitrogen (N). Total phosphorus (P) was measured after complete destruction of the soil samples with HClO₄ (65%), HNO₃ (70%) and H₂SO₄ (98%) in teflon bombs for 4 h at 150 °C. The concentrations of P were measured colorimetrically according to the malachite green procedure (Lajtha, Driscoll, Jarrell, & Elliott 1999). Bioavailable phosphorus (Olsen P) was measured by using extraction in NaHCO₃ (according to ISO 11263:1994 (E)) and colorimetric measurement according to the malachite green procedure (Lajtha et al. 1999). Potassium (K), calcium (Ca), magnesium (Mg) and aluminum (Al) were...
measured by extracting soil samples with NH₄ Ac-EDTA and by analyzing with atomic absorption spectrophotometry. Soil pH-H₂O was measured after mixing 10 g of soil and 50 ml of water and shaking for 5 min at 300 rpm using a pH meter Orion 920A (with pH electrode model Ross sure-flow 8172 BNWP, Thermo Scientific Orion, USA). More information can be found in Appendix A: Table S3 in Supplementary material.

Large-scale environmental conditions

We calculated mean annual temperature and precipitation at the scale of 30 arc-seconds (approximately 1 km²) using WorldClim version 2 (http://worldclim.org/version2) (Fick & Hijmans 2017). Atmospheric N deposition at each sampling site was calculated for the year 2015 as the sum of wet and dry depositions of oxidised (NOₓ) and reduced (NH₃) N based on modelled EMEP deposition data; and the model results of the 2016 version (data edition 2015v2016, 50 km resolution: http://www.emep.int/mscw/mscw_ydata.html#NCDATA; see Appendix A: Table S4 in Supplementary material).

Data analysis

All data were analysed in R version 3.4.3 (R Core Team 2017). To explore the data, we calculated Spearman correlations of pairs of variables in soil chemistry and large-scale environmental conditions (see Appendix A: Fig. S1 in Supplementary material) using the function cor in the corrplot package (Taiyun & Viliam 2017). We then used linear mixed-effects models (site nested within region as random factor) to test chemical soil differences between the four plant species (see Appendix A: Table S5 in Supplementary material) as well as between ancient and recent forests (see Appendix A: Table S6 in Supplementary material). Models were generated by using the function lmer in the package lme4 (Bates, Machler, Bolker, & Walker 2015). Model comparisons were tested by using maximum likelihood estimation. Data of chemical soil variables were log- or sqrt-transformed to meet the normality assumption of the statistical tests. We did not include latitude and longitude in our data analyses because spatial autocorrelation tests by using Mantel correlograms were not significant.

To test the compositional differences of soil microbial communities between the four plant species (the first hypothesis), we used non-metric multidimensional scaling (NMDS) with 999 runs using the function metaMDS (distance = Bray–Curtis) in the vegan package (Oksanen et al. 2016). The stress plot is shown in Appendix A: Fig. S2 in Supplementary material. The distinction was tested using PERMANOVA and pairwise PERMANOVA. To demonstrate which PLFA biomarker differs between the four plant species, we applied linear mixed-effects models (site nested within region as random factor) and multiple pairwise comparisons on each model using function glht (Tukey contrasts) in the multcomp package (Torsten, Frank, & Peter 2008) (see Appendix A: Table S7 in Supplementary material). Data of each PLFA biomarker were log- or sqrt-transformed to meet the normality assumption of the statistical tests.

To quantify the explanatory power of soil chemistry, large-scale environmental conditions and land-use history (three data groups) for the soil microbial community composition (the second hypothesis), variation partitioning was applied for each plant species based on redundancy analysis (RDA). First, the PLFA data was Hellinger transformed as this transformation produces more accurate estimates of R² values (Peres-Neto, Legendre, Dray, & Borcard 2006). Then, variation partitioning with three explanatory factors, i.e., soil chemistry, large-scale environmental conditions and land-use history, was applied to each plant species using the function varpart (package vegan). Additionally, an extra variation partitioning was applied to all plant species to illustrate the explanatory power of plant species identity in the variation of soil microbial community composition (see Appendix A: Fig. S3 in Supplementary material). We used adjusted R² values to express explained variations because of the unbalanced numbers of variables in different explanatory factors, and the significance of each factor’s explanatory power was tested using the function anova.cca.

To find the specific abiotic effects on soil microbial community composition under different plant species (the third hypothesis), we first used forward selection on all chemical soil variables in each plant species and retained the significant soil variables. Then, the significant soil chemistry variables were used in RDA for each plant species to assess their correlations with each PLFA biomarker. We plotted the correlation of the significant soil chemistry variables with each PLFA biomarkers using species and biplot scores from RDA (function rda in package vegan) by using package ggplot2 (Wickham 2009).

Results

Differences of soil microbial communities among plant species

The composition of the soil microbial community represented by PLFA biomarkers differed significantly among plant species (Fig. 2, P < 0.001). Pairwise PERMANOVA showed that the soil microbial community composition under *M. effusum* differed significantly from the other three plant species (see Appendix A: Table S8 in Supplementary material). At the biomarker level, there were 24 biomarkers showing significant differences among plant species (see Appendix A: Table S7 in Supplementary material). For instance, aC16:0 was absent in the soils of *M. effusum*, C24:1ω15c only occurred in the soils of *P. nemoralis*, and 16:1ω7t only occurred in the soils of *P. nemoralis* and *S. sylvatica* (and in one soil of *G. urbanum*).
Fig. 2. Composition of the soil microbial community based on PLFAs and analysed with non-metric multidimensional scaling (NMDS) of all PLFA biomarkers for four species (the distance metric was the Bray–Curtis metric). The stress value is 0.17. Ellipsoid hulls were added to enclose all points in each plant species. The significance test was based on a PERMANOVA.

Factors determining soil microbial community composition within each plant species

Across the four understorey species, soil chemistry explained more variation in the soil microbial community composition than the large-scale environmental conditions and land-use history (Fig. 3). For the grasses, both soil chemistry and large-scale environmental conditions significantly explained the variation in soil microbial community composition. The joint explanation by the two factors was 0.10 in *M. effusum* and 0.30 in *P. nemoralis*. The pure explanation by soil chemistry in the two grasses accounted for 0.27 in *M. effusum* and 0.21 in *P. nemoralis*. For the two forbs, only soil chemistry significantly explained the variation in soil microbial composition, and the pure explanation by soil chemistry amounted to 0.44 in *G. urbanum* and 0.36 in *S. sylvatica*. Land-use history did not significantly explain any of the variation in soil microbial community composition.

Correlations between soil chemistry and PLFA biomarkers

Across the four understorey species, the chemical soil variables that contributed significantly to the explanation of the variation in soil microbial communities were soil K, Ca, Mg, Al, Olsen P and pH, which were associated with biomarkers representing different microbial functional groups (Actinobacteria, non-specific bacteria, Gram-positive and Gram-negative bacteria and fungi) (Fig. 4 and Appendix A: Fig. S4 in Supplementary material). In *G. urbanum*, soil Olsen P, Mg and Al concentration were the significant soil variables correlated with the soil microbial community composition. The biomarkers of non-specific bacteria and fungi were positively correlated with Olsen P, while biomarkers of *Actinobacteria*, Gram-positive bacteria and Gram-negative bacteria (C18:1ω7t) were negatively correlated with Olsen P concentration. Gram-negative bacteria (C16:1ω7c) and

Fig. 3. Variation in soil microbial community composition explained by three factors, i.e., soil chemistry (Soil), large-scale environmental conditions (Env) and land-use history for each plant species. Adjusted $R^2$ values in each fraction indicate the explained percentage of the variation. Residuals indicate the unexplained variation. Adjusted $R^2$ values may cause small negative values. Asterisks show the significance of the permutation tests for each explanatory factor. *$P<0.05$, **$P<0.01$, ***$P<0.001$. 

fungi were positively correlated with soil Mg concentration, but negatively correlated with soil Al concentration. In *M. effusum*, soil Ca concentration and pH were significantly correlated with the soil microbial communities. Gram-negative bacteria (C16:1ω7c) were positively related to soil Ca concentration; Gram-positive bacteria (iC16:0) were negatively related to Ca concentration and soil pH. In *P. nemoralis*, five soil variables significantly predicted the soil microbial community composition, i.e., P, Olsen P, Ca, Al and pH. Gram-negative bacteria (C16:1ω7c) were positively correlated with Olsen P, while Gram-negative bacteria (C18:1ω7t) showed a negative relationship with Olsen P. In *S. sylvatica*, the composition of soil microbial communities significantly correlated with soil K and Al concentration, which were mostly driven by non-specific bacteria (C16:0) and Gram-positive bacteria (iC15:0 and iC16:0).
Discussion

We conducted an observational study on soil microbial community composition in root zone soils under four understory plant species (M. effusum, P. nemoralis, G. urbanum and S. sylvatica) across Europe. The four plant species harboured different soil microbial communities. Most importantly, our study was designed to assess the effects of abiotic factors in driving microbial community composition within plant species across Europe. Soil chemistry and large-scale environmental conditions, but not land-use history, were the main determinants of the root zone soil microbial community composition across all plant species. Particular chemical soil variables showed correlations with specific soil microbial communities, and these soil variables were mostly indicators for soil acidity.

Soil microbes differed among plant species

Confirming our first hypothesis, soil microbial community composition under the studied plant species differed significantly between M. effusum and the other three plant species. The importance of plant species in determining soil microbial community composition is also reflected by the significant explanation of plant species in the variation partitioning of the soil microbial community composition (see Appendix A: Fig. S3 in Supplementary material). This finding is consistent with many studies confirming the ecological linkages between aboveground plants and belowground microbes (Wardle, Yeates, Williamson, & Bonner 2003; Philippot, Raaijmakers, Lemanceau, & van der Putten 2013; Burns, Anacker, Strauss, & Burke 2015). Some detected biomarkers even only occurred under specific plant species, e.g., the fatty acid C15:1ω10c (not in P. nemoralis) and C24:1ω15c (only in P. nemoralis). In addition, the assessed biomass of each biomarker differed significantly among plant species, probably due to different root exudates and allelochemicals (Martinez-Garcia et al. 2015) between plant species. Next to rhizodeposition (e.g., root cap and border cell loss, root exudates), litter inputs by understorey plants are also important ways to connect plant species identity and soil microbial communities. Understorey plants contribute up to 20% of foliar litter input to the forest floor (Muller 2003), and litter chemical traits of different resources are related to the decomposition rate, thereby driving the function and composition of soil decomposers.

Three explanatory factors for soil microbial community composition

Our second hypothesis that soil chemistry, large-scale environmental conditions and land-use history are significant factors in determining soil microbial community composition in each plant species was partly supported. We only found that soil chemistry significantly determined soil microbial community composition within each plant species. Its explanation power ranged from 21% to 44% across the four study plant species. The significant influence of soil chemistry on microbial community composition is congruent with the previous study at a smaller spatial scale, which focused on the chemical soil conditions of forest tree (birch) in regulating the bacterial and fungal community composition (Mitchell et al. 2010). Soil chemistry includes many components and its influences on soil fungi and bacteria via, for instance, soil enzymes and microbial activity can ultimately lead to compositional shifts in soil microbial communities (Schappe et al. 2017; Waldrop et al. 2017). In turn, chemical soil conditions can also be modified through the activities of different soil microbes (Souza-Alonso, Novoa, & Gonzalez 2014).

The large-scale environmental conditions (climate and N deposition) explained a significant part of the soil microbial community composition of the two studied grasses (M. effusum and P. nemoralis). Mean annual temperature and N deposition are both positively correlated with mean annual precipitation across the studied latitudinal gradient (see Appendix A: Fig. S1 in Supplementary material). Changes in precipitation regimes have been considered as one of the main factors of changes in soil microbial community composition and diversity (Brockett et al. 2012). Less precipitation benefits the assembly of Gram-negative bacteria and fungi, while higher precipitation may result in more Gram-positive, anaerobic and sulphate-reducing bacteria (Drenovsky, Steenwerth, Jackson, & Scow 2010). Even the historical precipitation regime matters for the contemporary dynamics of the microbial community and therefore the biogeochemical cycling (Evans & Wallenstein 2012). The large-scale environmental conditions were not important for the soil microbial community composition in the two forbs we studied (G. urbanum and S. sylvatica). Given the inconsistent responses to environmental conditions for the soil microbial communities in the four studied plant species, more studies are needed to understand the determinants of large-scale environmental conditions on soil microbial community composition harboured by different host plants and ecosystems. The joint explanation, which can be explained by both soil chemistry and large-scale environmental conditions together, was 23% in P. nemoralis suggesting a strong correlation between the two factors in this species. Large-scale environmental conditions can affect soil microbial community indirectly through changes in soil chemistry. For instance, increased N deposition can decrease the concentrations of soil base cations (Shi et al. 2018). We indeed observed a negative correlation between N deposition and soil Mg concentrations (see Appendix A: Fig. S1 in Supplementary material).

Surprisingly, we found no significant effect of land-use history on soil microbial communities, and thus microbial community composition was found to be similar in ancient and recent forests in our study. This finding contradicts some previous studies (Ma, Guo, Lu, Yuan, & Wang 2015; de la Pena et al. 2016) which demonstrated that land-use legacies (mainly soil N and P concentration) affect the composition
and activity of soil microbial communities. In the study of Ma et al. (2015), soil microbial community composition in soil cores (5 cm diameter and 0–15 cm depth) was assessed at a regional scale in Northeastern China and they found that land-use change but not soil chemistry was one of the main factors in determining soil microbial community composition. Yet, our study corroborates the results of Jangid et al. (2011), who found that the soil microbial communities in soil cores (5 cm diameter and 0–10 cm depth) were similar in ancient forests and post-agricultural forests which had been established ca. 60 years ago. In our study, recent forests (except the Polish sites) had been established in the 19th century and were even up to 200 years old. Such long periods after land-use change may be enough for the recovery and accumulation of soil microbes and thus explain the similarity of soil microbial community composition between ancient and recent forests. Additionally, the four study species occurred both in recent and ancient forests, which partly support the absence of microbial composition difference between the two types of forests.

Admittedly, there were unexplained variations in soil microbial community composition in our study, suggesting that other potential factors should be included, for instance, tree species composition, plant traits and historic management.

The correlation between soil microbial community and soil acidity

Soil pH, Ca, and Al concentration, which are indicators for soil acidity, and Olsen P were among the soil chemical variables correlated with soil microbial community composition. Thus, our third hypothesis was supported. Soil microbial activities, such as microbial catabolic diversity (microbial responses to the addition of C-rich substrates), can be driven by soil pH and Ca concentration (Gartzia-Bengoetxea, Kandeler, de Arano, & Arias-Gonzalez 2016). Assessing soil microbial activity (although not available in this study) can facilitate the understanding of biochemical cycling and soil micorbial community dynamics and could be the focus of future research. In our study, soil Al concentration showed a negative relationship with Gram-negative bacteria in three species (G. urbanum, P. nemoralis and S. sylvatica). Free Al ions are toxic and may affect the effectiveness of biochemical cycling; Gram-negative bacteria can drive the decompositional efficiency of cellulose, hemicellulose, starch and monophosphoesters because of their potential influences on the catalytic properties of soil enzymes (Tischer, Blagodatskaya, & Hamer 2015). The correlations between soil microbes and Olsen P mostly varied between G. urbanum and P. nemoralis, but consistent correlation trends in both plant species did occur, for instance, between soil P and Gram-negative bacteria (C18:1ω7t, negative correlation) and fungal PLFAs (C18:2ω6c and C18:3ω3c, positive correlation). Interestingly, we observed a similar concentration range in Olsen P (19.7–51.6 mg kg⁻¹ for G. urbanum and 17.8–52.7 mg kg⁻¹ for P. nemoralis) (see Appendix A: Table S3 in Supplementary material). Fungi and Gram-negative bacteria are both involved in P solubilisation and mineralization (Chatí, Beri, & Sidhu 2008). Sufficient Olsen P may ease resource competition between fungi and Gram-negative bacteria (C16:1ω7c), but Gram-negative bacteria (C18:1ω7t) will be inhibited by higher soil P concentrations under G. urbanum and P. nemoralis, which implies a threat to microbial composition changes in P-eutrophic forests (Liu, Gundersen, Zhang, & Mo 2012).

Conclusion

Soil microbial community composition in the plant root zone varied substantially between plant species, as well as within populations of conspecifics at different latitudes. Chemical soil characteristics, particularly soil acidity and bioavailable phosphorus, along the studied latitudinal gradient were most important explaining the distinct microbial community composition under each plant species, suggesting an important role of local adaptation, inter- and intraspecific variation in understory plants. Recovery with respect to soil microbial community diversity in post-agricultural lands might be expected after several decades because plants established gradually and can facilitate the assemblage of soil microbes. With all these effects of abiotic and biotic factors on soil microbial community composition, we advocate the importance of maintaining the diversity of forest understory species as it can lead to a divergent soil microbial community composition. Our results also imply the possibility of using biogeochemical conditions to predict distribution patterns and dynamics of soil microbes across the globe and when facing environmental changes.

Author contributions

S.M., K.V. and P.D.F. planned and designed the research. S.M., P.D.F., J.B., S.A.O.C., G.D., A.K., I.L., J.L., T.N., A.O., J.P. and M.W. conducted fieldwork; S.M., P.D.F. M.V. and S.W. analysed the data; all authors contributed to the writing of the manuscript.

Acknowledgments

We thank L. Willems and G. De bruyn, S. Bodé and E. Gillis for laboratory assistance. This work was supported by China Scholarship Council (CSC) to S.M and by the Research Foundation – Flanders (FWO) for funding the scientific research network FLEUR (www.fleur.ugent.be). KV was supported by an ERC Consolidator Grant (PASTFORWARD; Grant no: 614839) and PDF by an ERC Starting Grant (FORMICA; Grant no: 757833).
Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.baae.2019.03.001.

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