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Effects of anthropogenic disturbances on soil microbial communities in oak forests persist for more than 100 years

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ABSTRACT

Land-use change and land-use intensification are considered amongst the most influential disturbances affecting forest diversity, community structure, and forest dynamics. Legacy effects of land-use changes in ecosystem functioning and services may last several hundred years. Although numerous studies have reported the short-term legacy effects of past management, analyses of long-term responses (>100 years) are still lacking. Here, we demonstrate shifts in soil microbial community structure and enzymatic activity levels resulting from a long-term past disturbance intensity gradient in oak forests (former arable farming – former heathland farming – ancient forest). Differences in microbial community composition among sites with contrasting historic land-use were related to differences in soil chemical properties and abundances of arbuscular mycorrhizal fungi, saprotrophic and ectomycorrhizal fungi, and actinobacteria. Both microbial biomass and enzymatic activity levels were distinctly lower in ancient forests compared to historically cultivated sites (i.e. agriculture or heathland farming). We found evidence that past land-use has long-lasting impacts on the recovery of soil community development, much longer than commonly assumed. This in turn highlights the importance of ecological continuity for ecosystem functioning and services. Conservation management, focussing on the stability and diversity of forest ecosystems, therefore needs to consider past land-use legacies for evaluating ecosystem functions (such as soil ecological processes) and for evaluating effective strategies to adapt to environmental changes.

1. Introduction

Interactions between belowground and aboveground communities may strongly influence ecosystem functioning by regulating plant community dynamics and biogeochemical processes (Wardle et al., 2004a; Wurzburger and Hendrick, 2009; Mangan et al., 2010). Belowground, soil microbial communities decompose organic materials, mediate carbon and nitrogen cycling, and determine nutrient availability for plant growth (Sparling, 1997; Aubert et al., 2010). Aboveground plant communities significantly alter microbial community composition and functions through rooting patterns, rhizodeposition, water use, litter chemistry, canopy structure, and subsequent influences on soil properties and microclimate (Bauhus et al., 1998; Weintraub et al., 2007; Huang et al., 2008; Zhang et al., 2009; Aubert et al., 2010; Burton et al., 2010; Wu et al., 2012). An important caveat is that above–belowground relationships are mediated by local edaphic factors, and thus such factors should be taken into consideration when assessing these relationships (Boyle et al., 2008; Wu et al., 2012).

Land-use changes can significantly alter the soil characteristics and aboveground species dynamics from which above- and belowground interactions develop (Lauber et al., 2008). Such land-use driven changes have been recognized as a main factor altering ecosystem functions, including carbon (C) and nitrogen (N) cycling or plant species diversity and productivity (Koerner et al., 1997; Brunet and von Oheimb, 1998; Guo and Gifford, 2002; Wakelin et al., 2009; Baeten et al., 2010; Cusack et al., 2013). Numerous studies have reported impacts of land-use changes on soil microbial communities (Fraterrigo et al., 2006; Lauber et al., 2008; Burton et al., 2010; Jangid et al., 2011), and microbial successional changes are increasingly used as an indicator of ecosystem recovery after anthropogenic disturbances (Harris, 2003; Banning et al., 2011). Land-use changes also influence microbial community structure and function and, consequently, nutrient cycling rates (e.g., Grayston and Rennenberg, 2006; Potthast et al., 2012; Ramirez et al., 2012).

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The persistence of these responses to land-use change is, however, still debated. Some chronosequence studies along successional gradients have shown that microbial communities tend to become more similar to those in native soils over time (Buckley and Schmidt, 2003; Jangid et al., 2010, 2011). However, there have been significant differences observed in microbial communities even after >50 years of conversion from agricultural cultivation to forests (Fraterrigo et al., 2005, 2006). To our knowledge, no study has investigated land-use legacies on microbial communities >100 years after afforestation. This is even though forest soils may continue to reflect their agricultural history for a far longer period, hypothetically through changes in soil chemical and structural properties (e.g. Verheyen et al., 1999; Compton and Boone, 2000; Jussy et al., 2002). Specifically, historical farming in temperate climates has resulted in higher soil phosphorous contents and lower soil carbon and nitrogen contents compared to sites with a long continuity of forest cover and soil development (Koerner et al., 1997; Dupouey et al., 2002; Fraterrigo et al., 2005; von Oheimb et al., 2008). Because microbial adaptation and recovery may play a significant role in ecosystem responses to human impacts (Mummey et al., 2002; Allison et al., 2010; Wallenstein and Hall, 2012), the long-term consequences of past land-use decisions on soil microbial communities are crucial for predicting changes in ecosystem functioning and services (Flinn and Vellend, 2005; Sun et al., 2011).

Here we examine the impact of land-use history on microbial community composition and functioning after more than 110 years of forest re-growth on former agricultural land. Very often, geographical past land-use patterns and environmental variation can be confounded (e.g. the fact that steeper slopes or poorer soils are more likely to be abandoned; Flinn and Vellend, 2005). To avoid this issue, we examined plots of sessile oak (*Quercus petraea*) dominated stands in the Lüneburg Heath region of Northwestern Germany that is characterized by homogeneous topography and soil conditions (Westphal, 2001). In addition, the land-use history in this region has been well documented over the last 240 years and detailed data on current site characteristics are available (Westphal, 2001; von Oheimb et al., 2008). Thus, we were able to select sites with very similar characteristics and tree species composition, but with different land-use histories. A previous study performed to determine past land-use effects on the edaphic properties of these forests (von Oheimb et al., 2008) showed that past agricultural practices resulted in long-term changes in essential soil characteristics, whereas differences between former heathlands and sites with a continuous forest history (ancient forest sites) were less pronounced.

Based on the previous research in these forests we hypothesized that different past land-uses would also have long-term impacts on microbial community composition and microbial extra-cellular enzyme activity. Specifically, we expected that long-term impacts on microbial communities would be strongest in former arable land compared to ancient forests, mirroring legacy effects of soil conditions related to past land-use intensity. Thus, our objective was to assess potential long-term effects of past land-use on (i) soil chemical properties, (ii) microbial biomass and community structure, and (iii) microbial extra-cellular enzyme activities. Furthermore we (iv) discuss the extent to which past land-use practices may have altered the recovery or trajectory of soil community development based on the results of our study. In order to measure microbial biomass and broad community structure simultaneously, we chose to use lipid analysis. Lipid analysis is a well-established method for quantitatively assessing microbial biomass and broad microbial groups including different fungal and bacterial communities (Vestal and White, 1989); it is also an effective measure of microbial responses to land-use and human impacts (for example: Mummey et al., 2002; Fraterrigo et al., 2006; Williams, 2007; Kulmatiski and Beard, 2011; Gutknecht et al., 2012). Microorganisms produce extra-cellular enzymes in order to degrade complex organic substrates into monomers for nutrient acquisition (Keeler et al., 2009). Extra-cellular enzyme activities can, therefore, represent microbial nutrient limitation and decomposition potential in response to changes in soil quality or land-use change (Sinsabaugh et al., 2002; Rinkes et al., 2011).

2. Material and methods

2.1. Study area

This study was conducted in the Lüneburg Heath nature reserve (Lower Saxony, NW Germany; 53°15′N, 9°58′E, 70–150 m a.s.l), which comprises an area of 24,000 ha. The study area is characterized by a humid suboceanic climate with a mean annual precipitation of 811 mm and a mean annual temperature of 8.4 °C (Müller-Westermeier, 1996). The geological substrate is composed of fluvioglacial sandy deposits and drift sands from the Saale Ice Age. As a result of the high substrate acidity, the soils are mainly Podzols (Rode, 1999). The potential natural vegetation is acidophytic mixed beech–oak forest.

The landscape has changed markedly due to various human management practices over the past 240 years. While heathland was the predominant land-use type in the 18th century (80%), a decline in historical farming activities, accompanied by afforestation measures during the last century, resulted in increased forest cover across the area (currently, app. 60%). At present the forests are dominated by coniferous species (68% *Pinus sylvestris*, 14% *Picea abies*, 5% *Larix decidua*, 2% *Pseudotsuga menziesii*), while deciduous trees account for 11% (5% *Quercus petraea*, *Quercus robur*; 3% *Fagus sylvatica*; 3% *Betula pendula*; Westphal, 2001).

2.2. Study design and stand characteristics

The study was based on a past land-use intensity gradient, using data from 18 mature sessile oak stands (Table 1). We restricted the analysis to oak forests for several reasons. Because significant differences have been observed in the (chemical) properties of the upper soil layers under different tree species planted on former cultivated land (e.g. Bauhus et al., 1998; Grayston and Prescott, 2005; Wu et al., 2012), it is important to exclude this confounding factor by keeping the tree species composition constant. The vast majority of the ancient forest sites are near-natural mixed broadleaved forests with a high proportion of oak and beech. However, afforestation of former agricultural land with broadleaved tree species always involved oak, never beech. In total, about 20% of the area of the Lüneburg Heath nature reserve that was converted from arable land and heathland to forest during the period 1878–1998 is now dominated by oak trees (Ernst and Hanstein, 2001). Furthermore, within the framework of “close-to-nature forestry”, most of the coniferous forests have been converted to mixed forests by planting oak trees over the last four decades.

Past land-use intensity was characterized on the basis of historical land-use systems: agriculture, heathland farming, and forestry. Information regarding past land-use was derived from historical maps of the “Kurhannoversche Landesaufnahme” from 1776 to 1786 and a forest management plan of 1887. The resulting gradient comprised (i) ‘FA’ oak stands established on former arable land, (ii) ‘FH’ oak stands established on former heathland and (iii) ‘AF’ oak stands on ancient forest sites. Agricultural practices in the 19th century included tillage and manure application. Fertilizer inputs were generally modest, with N-inputs mainly originating

Table 1
Land-use history ('FA' former arable land; 'FH' former heathland; 'AF' ancient forests) and the main stand characteristics of the investigated oak (*Quercus petraea*) forests. The shrub and herb layer (mean cover 30–40%) was dominated by *Fagus sylvatica*, *Picea abies*, *Rubus fruticosus*, *R. idaeus*, *Vaccinium myrtillus*, *Dryopteris carthusiana* agg. and *Deschampsia flexuosa*.

	FA	FH	AW
Historical management activities	Ploughing, application of manure	Sheep grazing, sod-cutting	Firewood collection, litter ranking
Forest continuity (years)	app. 110	app. 120	>235
Stand age (years)[a]	111 (11)	117 (16)	139 (18)
Stand volume (m^3 ha^{-1})[a]	295 (19)	257 (76)	346 (65)
Species composition (%)[b]			
Quercus petraea	86	80	67
Fagus sylvatica	2	–	25
Pinus sylvestris	6	15	4
Picea abies	6	3	4
Other tree species	–	1	–
n (plots)	6	6	6

[a] Mean (SD).
[b] Mean proportion of canopy tree basal area according to the forest management plan of 2011.

from organic sources. In contrast, sheep grazing was the main use of heathlands during this period. In addition, regular removal of litter, used for indoor sheep bedding, resulted in a decrease in the soil fertility in heathlands. Subsequently, manure-enriched litter was transferred to the arable fields, thus serving as an organic fertilizer (see Gimingham, 1972 for a detailed description of historical heathland farming). Accordingly, the past land-use intensity decreases within the series FA – FH – AF. Forestry measures were restricted to selective logging (with no tillage). During 1981 and 1988, all study sites were ameliorated by liming (mean application: 3 t ha^{-1}).

2.3. Soil sampling and chemical analyses

In April 2011, we randomly collected five soil samples from the upper 5 cm mineral soil (A_{he}-horizon) for each study stand using a 100 cm^3 cylindrical metallic corer. The cores were taken a minimum distance of 5 m from each other and were immediately chilled. For subsequent analyses the five subsamples were thoroughly mixed to obtain one composite sample per stand. Total C, total N, total P, CEC and pH were determined from the homogenized soil samples. All samples were sieved (<2 mm), ground and dried at 105 °C prior to soil chemical analyses. Total C and N were determined with a C:N analyzer (Vario EL, Elementar, Hanau, Germany). For the determination of total P, samples were dissolved in an HNO_3–HCl–H_2O_2 solution (Wong et al., 1997) and digested using a microwave (MLS-ETHOS; MLS-GmbH, Leutkirch, Germany). Digested samples were analysed with an ICP-OES. Determination of CEC followed standard procedures as described by Steubing and Fangmeier (1992). Soil pH was measured in a 1:5 soil:water suspension.

2.4. Microbial lipid analysis

Lipid analysis was used to determine microbial biomass and microbial community structure. The applied procedure is a combination of phospholipid fatty acid analysis (PLFA, adapted from Bligh and Dyer, 1959) and fatty acid methyl ester analysis (FAME, modified from Gutknecht et al., 2012). From each composite soil sample, 2 g were extracted three times in a single phase citrate buffer (1.8 ml, 0.15 M), chloroform (2 ml), methanol (4 ml) mixture (0.9:1:2 volume ratio). After extraction, the volume ratio was changed to 0.9:1:1 to allow the phases to separate overnight at room temperature. The chloroform phase, containing the fatty acids, was retained and evaporated using a nitrogen evaporator. The procedure for FAME was then followed (Microbial ID Inc, Hayward CA); saponification followed by strong acid methanolysis and phase separation to extract the methyl-esterfied fatty acids. Methyl-esterfied fatty acids were run on a Gas chromatograph (Hewlett–Packard, HP 6890 Series GC-System) interfaced to a mass spectrometer (Agilent 5973) with an HP-5MS column (30 m, 0.25 m internal diameter, coated with a cross-linked 5% phenyl methyl rubber phase with a film thickness of 0.35 mm). Lipid peaks were determined manually using the associated Agilent FAME identification library, based on retention time, mass spectra, and comparison with standards. Peak areas were converted into nmol lipid g $soil^{-1}$ using the internal standard C 19:0, and the efficiency of extraction was determined using a 13:0 surrogate standard added to each sample and blanks at the beginning of extractions.

The total nmol lipid g $soil^{-1}$ (sum of all lipids present, 20 or less carbons in length) was used as an index of microbial biomass (Vestal and White, 1989). In addition, chemically similar lipid indicators were used to represent ecological groups of microorganisms. These included the following: Gram+ bacteria (sum of 13:0 iso, 13:0 anteiso, 14:0 iso, 15:0 iso, 15:0 anteiso, 16:0 iso, 17:0 iso, 17:0 anteiso, 18:0 iso and 18:0 anteiso), Gram– bacteria (sum of 10:0 2OH, 14:1, 14:0 2OH, 14:0 3OH, 16:1 ω9c, 16:1 ω7c, 16:1 ω7t, 17:1 11c or 9c, 17:1 7c or 8c, 16:0 2OH, 16:0 2OH, 16:1 OH, 18:1 ω12c, 18:1 ω9t, 18:1 ω7c, 18:1 ω5c, 18:1 2OH and 19:1), actinobacteria (sum of 16:0 10me, 16:0 me B, 16:0 11me, 17:0 me A, 18:1 me, 18:0 10me and 18:0 12me), anaerobic bacteria (17:0 cyclo and 19:0 cyclo), arbuscular mycorrhizal (AM) fungi (16:1 ω5c) as well as saprotrophic and ectomycorrhizal (S-EM) fungi (18:2 unknown, 18:2 ω6,9c and 18:1 ω9c; Balser et al., 2005). The ratios of fungal:bacterial lipids (f:b ratio) and Gram+ bacterial:Gram– bacterial lipids (G+:G– ratio) were also included in the data analysis.

2.5. Microbial extra-cellular enzyme activity

The activity of microbial extra-cellular enzymes was analysed according to German et al. (2011). Soil sample suspensions were prepared by adding 1.0 g soil to 100 ml of 2.5 M TRIS buffer (pH 7) and homogenizing for 5 min by low-level sonication. From the resulting suspensions, 200 μl were added to 96-well microplates containing 50 μl of fluorescent MUB (4-Methylumbelliferone) linked substrates to test the activity of three extra-cellular enzymes: phosphatase, β-glucosidase, and N-acetylglucosaminidase. The final concentrations of substrate solutions were 150 μM for phosphatase and β-glucosidase and 200 μM for N-acetylglucosaminidase. Substrate concentrations and incubation times were determined by preliminary analysis of representative soil samples from our study sites. Each 96-well plate also contained substrate

controls (200 µl buffer and 50 µl of substrate), homogenate controls (200 µl of sample suspension and 50 µl of buffer), a dilution series to determine the sample quench coefficient (MUB and 200 µl sample suspension) and a dilution series to determine the emission coefficient (MUB and 200 µl TRIS buffer). The microplates were incubated at room temperature in the dark for one hour. To stop the reaction and for fluorescence measurements, 10 µl of 0.5 M NaOH was added after incubation to every well of each microplate. Fluorescence was measured using a Microplate Reader (Fluorescence Spectrophotometer VARIAN Cary Eclipse). Using the fluorescence of assay wells, controls, and the quench and emission coefficients, activities were calculated as nmol substrate cleaved g soil^{-1} h^{-1} as described by German et al. (2011).

2.6. Data analysis

Variation in microbial community assembly with land-use history was evaluated by analysis of dissimilarity (ADONIS, 1000 permutations) followed by a Bonferroni adjustment (Anderson, 2001). The analysis was performed on a matrix of Bray–Curtis dissimilarities based on the relative mole fractions of individual lipid markers ($n = 36$). Abundance data were square-root transformed and standardized (Wisconsin double standardization) prior to analysis. Only fatty acids >0.5 mol percent were considered for the analysis. The same matrix was also used for non-metric multidimensional scaling (NMDS, using the *metaMDS* function of the *vegan* library in R, Oksanen et al., 2011) in order to reveal patterns in microbial composition among the past land-use types. To examine how microbial communities vary along the ordination axes, we calculated Pearson correlation coefficients between microbial groups and NMDS-axis scores. Similarly, Pearson correlation tests were applied to explore changes in the community structure with chemical soil properties. Finally, the microbial groups and soil parameters with significant Pearson axes correlations were included in the ordination plot using the function *envfit* in the *vegan* library in R. The length and direction of the vectors indicate the strength and direction of their correlation with NMDS-axis scores.

Differences between past land-use types and chemical soil properties, microbial abundance (untransformed data) and enzyme activities ($\log_{10}$-transformed data) were tested by ANOVA with a post hoc Tukey HSD test. To investigate the relationship between enzyme activities and microbial groups or total biomass we applied multiple linear regressions. The variable f:b ratio was omitted from the analysis because of its close correlation with the abundance of saprotrophic and ectomycorrhizal fungi ($r = 0.85$). The explanatory power of significant predictors was assessed by variance partitioning based on adjusted R^2 values (Zuur et al., 2007). All analyses were performed with R, version 2.14.2. (Team RDC, 2012).

3. Results

3.1. Soil chemical properties

Chemical soil properties varied markedly between the three land-use types. Total C, N and P strongly decreased from FA/FH to AF, while C:N ratios were significantly higher (+37%) at the AF sites compared to the FA sites (Table 2). Past land-use effects on pH and CEC were less distinct.

3.2. Multivariate community composition

The NMDS ordination resulted in a two-dimensional solution with a final stress of 0.120. Microbial community development was strongly affected by past land-use intensity (ADONIS: $F = 2.47$, $P < 0.001$). Community composition in AF soils differed markedly from FA ($P_{adj.} < 0.01$), while a marginal difference ($P_{adj.} = 0.04$) was noted for AF and FH. Differences between FA and FH were not statistically significant ($P = 0.23$). PLFA profiles of the land-use types were clearly separated along the first NMDS axis ($P = 0.017$), while along axis 2 no past land-use effect was evident ($P = 0.44$; Fig. 1). Moreover, apart from one outstanding FA and FH sample, AF samples were the most scattered across the ordination diagram, indicating a trend towards higher structural heterogeneity between microbial communities (higher β-diversity) with decreasing past land-use intensity. Variation in microbial assemblages showed a strong response to chemical soil properties. Axis 1 corresponded significantly to a nutrient gradient of increasing C:N ratios ($r = 0.58$) and decreasing N, P, C and CEC contents (N: $r = -0.78$; P: $r = -0.77$; C: $r = -0.71$; CEC: $r = -0.73$; Table 3 and Fig. 1). Along this axis, assemblage composition was driven in large part by the abundance of S-EM fungi ($r = 0.68$) and actinobacteria ($r = -0.63$; Table 3).

Table 2
Soil chemical characteristics (mean ± SE) of the study stands from the upper mineral soil (A_{he}-horizon). Different letters in online indicate statistically significant (Tukey HSD: $P_{adj.} < 0.05$) differences between the past land-use types. 'FA' former arable land; 'FH' former heathland; 'AF' ancient forests.

	FA	FH	AF
Total C (%)	2.54 (0.36)a	3.10 (1.00)a	1.41 (0.25)a
Total N (%)	0.13 (0.02)a	0.14 (0.05)a	0.05 (0.01)b
Total P (mg kg^{-1})	185.16 (17.34)a	135.50 (39.91)ab	76.83 (11.27)b
C:N ratio	19.48 (1.19)a	23.57 (1.17)ab	26.61 (1.74)b
C:P ratio	142.50 (20.43)a	220.50 (12.44)b	185.33 (17.25)ab
CEC (mval L^{-1})[a]	4.78 (0.63)a	5.43 (1.30)a	3.58 (0.40)a
pH	4.05 (0.20)a	3.94 (0.14)a	3.98 (0.17)a

[a] Cation exchange capacity.

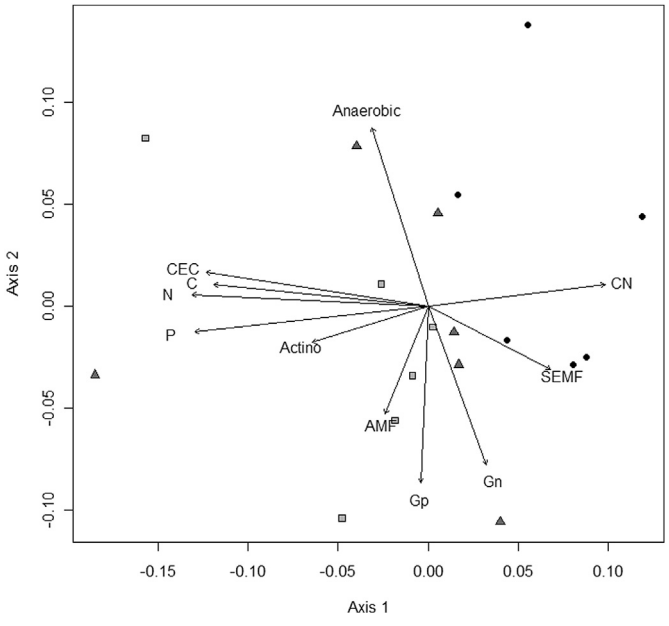

Fig. 1. Non-metric multi-dimensional scaling ordination of soil microbial communities in oak forests with different land-use histories: former arable land (light grey squares), former heathland (grey triangles) and ancient forests (black circles). Site scores ($n = 18$) represent microbial assemblages in the A-horizon. Arrows indicate significant ($P < 0.05$) joint axis correlations with microbial groups (AMF: arbuscular mycorrhizal fungi; SEMF: saprotrophic and ectomycorrhizal fungi; Actino: actinobacteria; Anaerobic: anaerobic bacteria, Gn: Gram− bacteria; Gp: Gram+ bacteria) and soil properties (C: total carbon content, N: total nitrogen content, P: total phosphorous content; CEC: cation exchange capacity; CN: C:N ratio).

Table 3
Correlation matrices of associations between NMDS axes scores, and microbial groups and chemical soil properties for 18 mature sessile oak (*Quercus petraea*) stands. Values indicate Pearson coefficients, significant correlations are in bold. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

	Axis 1	Axis 2
Microbial groups		
Gram+ bacteria	−0.047	**−0.841*****
Gram− bacteria	0.367	**−0.733*****
Anaerobic bacteria	−0.355	**0.832*****
Actinobacteria	**−0.633****	−0.145
AM fungi[a]	−0.268	**−0.494***
S-EM fungi[b]	**0.681****	−0.259
G+:G- ratio	−0.398	0.962
Fungi:bacteria ratio	0.402	−0.254
Soil characteristics		
pH	−0.005	−0.264
Total C	**−0.707****	0.054
Total N	**−0.777*****	0.029
Total P	**−0.770*****	−0.061
C:N ratio	**0.585****	0.053
C:P ratio	−0.001	−0.026
CEC[c]	**−0.735*****	0.084

[a] AM arbuscular mycorrhiza.
[b] S-EM saprotrophic and ectomycorrhizal.
[c] Cation exchange capacity.

3.3. Microbial biomass and broad groups

Historical farming had a marked impact on the distribution of microbial groups. In total, FH soils were associated with the highest microbial biomass, followed by FA (−23%) and AF (−44%; Fig. 2). However, differences were not statistically significant. This can be mainly attributed to the considerable variation within FH and FA plots. Values for the coefficient of variation accounted for 52.4% (FH) and 30.3% (FA) compared to 21.9% (AF). On average, AF soils supported a 25% and 8% lower proportion of actinobacteria compared to FH and FA samples, respectively. AF soils, on average, also supported a 52% and 40% lower proportion of AM fungi compared to FH and FA samples. In addition, mean values of S-EM fungi and anaerobic bacteria tended to increase with decreasing past land-use intensity, but showed no significant differences among land-use types. The opposite trend was observed for Gram+ bacteria (Table 4).

3.4. Microbial enzyme activities

Past land-use intensity significantly affected enzyme activity (N-acetylglucosaminidase: F: 12.29, $P < 0.001$; β-glucosidase: F: 6.11, $P < 0.05$; phosphatase: F: 3.58, $P < 0.01$) and was highest in FH, followed by FA and AF (Fig. 2). This pattern was consistent for all enzymes. Microbial activities in former arable land or heathland soils were, on average, up to five and nine times higher, respectively, than in ancient forest soils.

N-acetylglucosaminidase and β-glucosidase activities significantly increased with increasing total microbial biomass and abundance of AM fungi. Microbial biomass, however, was much more related to β-glucosidase than for N-acetylglucosaminidase activity. In contrast, phosphatase activity was positively related to actinobacterial abundance. No other microbial groups showed any significant ($P > 0.05$) relationships with enzyme activities (Table 5).

Table 4
Mean (±SE) site values ($n = 6$ per land-use type) for different microbial groups (mol %) in mature sessile oak (*Quercus petraea*) stands with contrasting past land-use intensities. Different letters in online indicate statistically significant (Tukey HSD: $P_{adj.} < 0.05$) differences between the past land-use types. 'FA' former arable land; 'FH' former heathland; 'AF' ancient forests.

	FA	FH	AF
Gram+ bacteria	8.36 (0.75)a	8.44 (0.54)a	7.13 (0.50)a
Gram− bacteria	26.82 (1.79)a	23.54 (3.20)a	24.82 (2.36)a
Anaerobic bacteria	21.63 (4.71)a	21.70 (2.61)a	24.10 (3.32)a
Actinobacteria	3.32 (0.14)a	4.10 (0.43)a	3.06 (0.19)b
AM fungi[a]	0.86 (0.11)ab	0.97 (0.11)a	0.52 (0.05)b
S-EM fungi[b]	5.92 (0.48)a	6.85 (0.42)a	7.13 (0.81)a
G+:G- ratio	0.31 (0.02)a	0.37 (0.04)a	0.30 (0.02)a
Fungi:bacteria ratio	0.12 (0.01)a	0.14 (0.01)a	0.13 (0.02)a

[a] AM arbuscular mycorrhiza.
[b] S-EM saprotrophic and ectomycorrhizal.

4. Discussion

4.1. Effects of past land-use on soil microbial community structure and enzyme activities

We found strong support for the hypothesis that past land-use, even after a century of reforestation, still influences soil microbial community composition and microbial extra-cellular enzyme activities. However, contrary to our hypothesis, we found no evidence for distinct differences in soil microbial community composition or enzyme activity between historical farming practices (between agriculture and heathland grazing). Specifically, we observed considerably higher microbial biomass and enzyme activities on former heathlands and former arable lands compared to ancient oak forest sites, but not between the historically cultivated sites. This strongly suggests that legacy effects of past land-use can be a

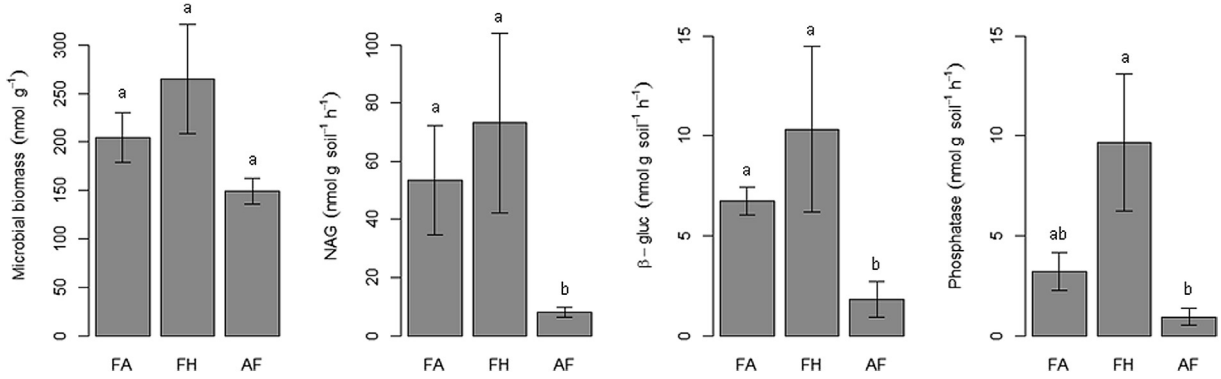

Fig. 2. Variation in mean (±SE) abundance of total microbial biomass (nmol lipid g soil^{-1}) and enzyme activities (nmol substrate cleaved g soil^{-1} h^{-1}) along a past land-use gradient. Different letters indicate statistically significant (Tukey HSD: $P_{adj.} < 0.05$) differences between the past land-use types. 'FA' former arable land; 'FH' former heathland; 'AF' ancient forests. NAG 'N-acetylglucosaminidase'; βgluc 'β-glucosidase'.

Table 5
Relationships between PLFA data (microbial groups and total biomass) and log–enzyme activity (nmol substrate cleaved g soil^{-1} h^{-1}) in 18 mature sessile oak (*Quercus petraea*) stands. The importance of the predictors is given by the partial R^2 derived from variance partitioning.

	N-acetylglucosaminidase			β-Glucosidase			Phosphatase	
	Effect	*P*-value	Partial R^2	Effect	*P*-value	Partial R^2	Effect	*P*-value
Gram+ bacteria		n.s.			n.s.			n.s.
Gram− bacteria		n.s.			n.s.			n.s.
Anaerobic bacteria		n.s.			n.s.			n.s.
Actinobacteria		n.s.			n.s.		+	<0.001
AM fungi[a]	+	<0.001	0.344	+	0.013	0.134		n.s.
S-EM fungi[b]		n.s.			n.s.			n.s.
G+:G− ratio		n.s.			n.s.			n.s.
Total microbial biomass	+	0.002	0.211	+	<0.001	0.386		n.s.
$R^2_{adj.}$		0.745			0.687			0.495

[a] AM arbuscular mycorrhiza.
[b] S-EM saprotrophic and ectomycorrhizal.

major driver of soil microbial community dynamics, but that the land use itself, and not necessarily the type of land use, may be important for these legacy effects.

Although very few studies have been able to assess persistent changes in soil conditions or soil communities resulting from land-use change after as long a time as were able to explore, our results contribute to a growing body of evidence that different land-use practices alter soil communities in the long term. This is in contrast to the common paradigm that microbial communities are infinitely plastic or can adapt to new conditions very rapidly (Schimel et al., 2007). Fraterrigo et al. (2006), for instance, found lower fungal abundance, higher bacterial abundance, and higher N-mineralization rates in forest stands 50 years after the stands had been reforested after agricultural cultivation. It has also been shown that bacterial communities continue to change over the course of 50–2000 years of rice cultivation, compared to non-cultivated soils (Bannert et al., 2011). In our study, we observed that arbuscular mycorrhizal fungi, actinobacteria, and enzyme activities were distinctly lower in ancient forest soils, the least fertile soils examined in our study, compared to recent forest soils (FA, FH). The former arable lands and heathlands that we examined had persistently more fertile soils with approximately two-fold higher concentrations of N and P (Table 2). Thus, in this case, past land-use has actually led to soils with higher long-term nutrient concentrations (von Oheimb et al., 2008), and thus the potential to support more growth and enzyme activity of those microbial groups. Contrarily, our observed trend towards higher abundances of saprotrophic and ectomycorrhizal fungi in ancient forest soils can be explained by higher amounts and structural complexity of carbon sources (dead wood or litter) in forests with a long continuity of low anthropogenic disturbances compared to recent forests (von Oheimb et al., 2007), leading to more niche availability for larger fungal communities. Together these results suggest that changes in edaphic site properties alter microbial community composition and consequent microbial activities to a much greater extent than land-use per se (Lauber et al., 2008; Birkhofer et al., 2012).

A caveat to our study is that the trajectory of ecosystem development after long-term land-use can often be confounded by current modified 'original' plant communities (Kulmatiski and Beard, 2008). In our case, tree species composition distinctly varied between past land-use types with 19% (FH) and 28% (FA) higher abundance of *Q. petraea* compared to ancient forest sites (AF). In contrast, the proportion of *F. sylvatica*, which is the second most common species in ancient oak forests, was much lower in former arable lands and former heathlands, respectively (Table 1). Sites where there was agriculture or heathland farming in the past, of course, have greater oak dominance because they were deliberately reforested by planting oak seedlings. Although we should be aware of this caveat to our study, there is also evidence that past land-use is more influential for soil microbial communities than current plant community composition (Jangid et al., 2011). Thus, the observed differences in soil characteristics and the associated persistent changes in the soil microbial communities could be directly related to the effects of historic land-use and also to subsequent feedbacks from the altered tree species composition.

The process of conversion from former heathlands to forests may also explain our observed differences in microbial extracellular enzyme activities and actinobacterial abundances. Heathland soils are typically dominated by dwarf shrub species (of the Ericaceae, such as *Calluna vulgaris*) that are high in polyphenolic compounds. These polyphenolic compounds inhibit decomposition and thus, organic matter breaks down slowly in heathlands and they have a high potential for carbon storage (Nielsen et al., 1987). In addition, heathlands were traditionally used for grazing, and there may have been organic carbon inputs (originating from the sheep) that were stored in the soil because of the low decomposition rates. However, after afforestation with oak, ericaceous dwarf shrubs disappeared, with a subsequent halt in the inputs of polyphenolic compounds. Over time (decades or centuries) this would lead to a greater capacity for decomposition, such as we observed with the increase in microbial biomass and extra-cellular enzyme activity. Another typical change in microbial communities after conversion of heathlands to forests is the shift in dominance from ericoid mycorrhizal fungi to ectomycorrhizal fungi. The ability of ericoid mycorrhizas to assimilate phenolic monomers and, by cleaving polyphenols, to secure the release of nutrients coprecipitated with these polymers is particularly relevant for the nutrition of ericaceous plants in heathlands (Read and Perez-Moreno, 2003). The conversion to oak forests and transition to an ectomycorrhizal based fungal community could explain the higher extra-cellular enzyme activities and actinobacterial abundance we observed. Ectomycorrhizal hyphae and associated mats are known to be associated with high levels of enzyme production (Pritsch et al., 2004; Kluber et al., 2010). This high level of enzyme production possibly comes directly from the fungi or from associated actinobacterial populations. The significant correlation we found between actinobacterial abundances and extra-cellular enzyme activities is thus logical given that actinobacteria are major producers of extracellular enzymes.

4.2. Effects of past land-use intensity on successional pathways of forest soil microbial communities

The magnitude and the direction of management effects may vary with ecosystem properties and past land-use intensity. There are two plausible, non-mutually exclusive, explanations for long-

term past land-use effects on both soil microbial communities and nutrient cycling. First, depending on the precise initial site and soil conditions, as well as on subsequent management practices, recovery of forest ecosystems back to pristine or undisturbed conditions could take more than 200 years. Thus, the observed shifts in soil conditions, tree species composition and microbial community structure in our study sites are still out of a long-term equilibrium state and continue to recover from past land-use impacts. Given the relatively slow process of soil formation, slow recovery rate of soil microbial communities (Jangid et al., 2011) and the long-lived dominant plant species in forests, it seems plausible that successional pathways can be long-lasting. This is consistent with the findings of von Oheimb et al. (in prep.), who observed, for the same long-term chronosequence study sites, considerably higher variation in tree-ring width and higher mean growth rates of *Q. petraea* on former agricultural and heathland sites than on ancient woodland sites. Additionally von Oheimb et al. (in prep.) found significant correlations of tree-ring width with C:N ratio and P availability, which indicates that past land-use continues to affect forest soil conditions and tree growth patterns for more than a century. In other studies it has also been documented that ecosystem development shows a consistent pattern of increasing and then decreasing soil fertility, with the early developmental phases lasting 200 or more years (Dupouey et al., 2002; Wardle et al., 2004b; Peltzer et al., 2010). For example Cusack et al. (2013) reported long-term (>200 years) effects of intensive cultivation on soil carbon pools and cycling. The recent forest communities (FA, FH) analysed in our study are, therefore, probably still in this early developmental, high fertility phase. There are associated changes in microbial community structure along this sequence of recovery, although they are relatively unpredictable or poorly understood (Dickie et al., 2013). Our observations indicated higher abundances of actinobacterial and arbuscular mycorrhizal fungal lipid indicators as well as total lipid biomass in both former arable lands and former heathlands when compared to ancient forests (as discussed earlier).

The second possible explanation is that, instead of a progression toward recovery or equilibrium, past land-use has actually changed the long-term trajectory and adaptation of both forest and soil community development over time. Even though microbial communities may be adapted to natural disturbance regimes (Gutknecht et al., 2010, 2012), human induced land-use changes could represent disturbance events that are beyond the adaptive capacity, or that change the adaptive capacity, of the community in question (Scheffer et al., 2009; Leadley et al., 2010). This is due to the drastic alterations in historic site conditions (e.g. tillage, fertilization and altered tree species composition) created by those land-use changes. Our results indicate that past land-use has led to higher nutrient levels and altered vegetation composition. Consequently, these changes in ecosystem properties may have shifted the equilibrium state of the microbial community, and further research should address the question of whether this leads to long-term increases in decomposition and nutrient cycling in these forest ecosystems. Thresholds such as long-term land-use may, therefore, alter edaphic properties to an extent that induces positive feedbacks and thus are hard to change further or reverse (Lenton et al., 2008; Scheffer et al., 2009; Leadley et al., 2010). Accordingly, even 'restored' native forest or soil communities can never again represent historic forest conditions because of legacy effects of past management.

4.3. Conclusions

In this study we found strong evidence that past land-use effects on forest soil microbial communities persist much longer than observed to date (e.g. Fraterrigo et al., 2005, 2006; Jangid et al., 2011). This in turn suggests that ecological continuity may be a major driver of ecosystem functioning and services. After more than one century of development during secondary succession, microbial community structure and enzyme activities were still significantly different between ancient and recent forest ecosystems. Thus, successional pathways of forest soil microbial communities probably depend on the intensity of past anthropogenic disturbance, since compositional shifts were less pronounced in former heathlands compared to former arable lands. This can be mainly attributed to differences in the initial soil conditions after anthropogenic disturbances.

Beyond the ability to generalize our results to other forest types, we conclude that a deeper understanding of various past-land-use legacies is crucial, because of their essential role for above- and belowground interactions. In this context, ancient forest sites are particularly important for the conservation of aboveground (e.g. Baeten et al., 2010) and belowground communities and thus, for the diversity and functioning of forest ecosystems.

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References

Allison, S.D., Wallenstein, M.D., Bradford, M.A., 2010. Soil-carbon response to warming dependent on microbial physiology. Nature Geoscience 3, 336–340.
Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Australian Journal of Ecology 26, 31–46.
Aubert, M., Margerie, P., Trap, J., Bureau, F., 2010. Aboveground-belowground relationships in temperate forests: plant litter composes and microbiota orchestrates. Forest Ecology and Management 259, 563–572.
Baeten, L., Hermy, M., Van Daele, S., Verheyen, K., 2010. Unexpected understorey community development after 30 years in ancient and post-agricultural forests. Journal of Ecology 98, 1447–1453.
Balser, T., Treseder, K., Ekenler, M., 2005. Using lipid analysis and hyphal length to quantify AM and saprotrophic fungal abundance along a soil chronosequence. Soil Biology and Biochemistry 37, 601–604.
Bannert, A., Kleineidam, K., Wissing, L., Mueller-Niggemann, C., Vogelsang, V., Welzl, G., Cao, Z., Schloter, M., 2011. Changes in diversity and functional gene abundances of microbial communities involved in nitrogen fixation, nitrification, and denitrification in a tidal wetland versus paddy soils cultivated for different time periods. Applied and Environmental Microbiology 77, 6109–6116.
Banning, N.C., Gleeson, D.B., Grigg, A.H., Grant, C.D., Andersen, G.L., Brodie, E.L., Murphy, D.V., 2011. Soil microbial community successional pattern during forest ecosystem restoration. Applied and Environmental Microbiology 77, 6158–6164.
Birkhofer, K., Schöning, I., Alt, F., Herold, N., Klarner, B., Maraun, M., Marhan, S., Oelmann, Y., Wubet, T., Yurkov, A., Begerow, D., Berner, D., Buscot, F., Daniel, R., Diekötter, T., Ehnes, R.B., Erdmann, G., Fischer, C., Foesel, B., Groh, J., Gutknecht, J., Kandeler, E., Lang, C., Lohaus, G., Meyer, A., Nacke, H., Näther, A., Overmann, J., Polle, A., Pollierer, M.M., Scheu, S., Schloter, M., Schulze, E.-D., Schulze, W., Weinert, J., Weisser, W.W., Wolters, V., Schrumpf, M., 2012. General relationships between abiotic soil properties and soil biota across spatial scales and different land-use types. Plos One 7. http://dx.doi.org/10.1371/journal.pone.0043292.
Bauhus, J., Pare, D., Cote, L., 1998. Effects of tree species, stand age and soil type on soil microbial biomass and its activity in a southern boreal forest. Soil Biology and Biochemistry 30, 1077–1089.
Bligh, E., Dyer, W., 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37, 911–917.
Boyle, S.A., Yarwood, R.R., Bottomley, P.J., Myrold, D.D., 2008. Bacterial and fungal contributions to soil nitrogen cycling under Douglas fir and red alder at two sites in Oregon. Soil Biology and Biochemistry 40, 443–451.
Brunet, J., von Oheimb, G., 1998. Migration of vascular plants to secondary woodlands in southern Sweden. Journal of Ecology 86, 429–438.

Buckley, D.H., Schmidt, T.M., 2003. Diversity and dynamics of microbial communities in soils from agro-ecosystems. Environmental Microbiology 5, 441–452.
Burton, J., Chen, C.R., Xu, Z.H., Ghadiri, H., 2010. Soil microbial biomass, activity and community composition in adjacent native and plantation forests of subtropical Australia. Journal of Soils and Sediments 10, 1267–1277.
Compton, J.E., Boone, R.D., 2000. Long-term impacts of agriculture on soil carbon and nitrogen in New England forests. Ecology 81, 2314–2330.
Cusack, D.F., Chadwick, O.A., Ladefoged, T., Vitousek, P.M., 2013. Long-term effects of agriculture on soil carbon pools and carbon chemistry along a Hawaiian environmental gradient. Biogeochemistry 112, 229–243.
Dickie, I.A., Martínez-García, L.B., Koele, N., Grelet, G.A., Tylianakis, J.M., Peltzer, D.A., Richardson, S.J., 2013. Mycorrhizas and mycorrhizal fungal communities throughout ecosystem development. Plant and Soil 367, 11–39.
Dupouey, J.L., Dambrine, E., Laffite, J.D., Moares, C., 2002. Irreversible impact of past land-use on forest soils and biodiversity. Ecology 83, 2978–2984.
Ernst, G., Hanstein, U., 2001. Epiphytische Flechten im Forstamt Sellhorn – Naturschutzgebiet Lüneburger Heide. NNA-Berichte 14, 28–85.
Flinn, K.M., Vellend, M., 2005. Recovery of forest plant communities in post-agricultural landscapes. Frontiers in Ecology and the Environment 3, 243–250.
Fraterrigo, J.M., Balser, T.C., Turner, M.G., 2006. Microbial community variation and its relationship with nitrogen mineralization in historically altered forests. Ecology 87, 570–579.
Fraterrigo, J.M., Turner, M.G., Pearson, S.M., Dixon, P., 2005. Effects of past land use on spatial heterogeneity of soil nutrients in southern Appalachian forests. Ecological Monographs 75, 215–230.
German, D.P., Weintraub, M.N., Grandy, A.S., Lauber, C.L., Rinkes, Z.L., Allison, S.D., 2011. Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biology and Biochemistry 43, 1387–1397.
Gimingham, C.H., 1972. Ecology of Heathlands. Chapman and Hall, London.
Grayston, S.J., Prescott, C.E., 2005. Microbial communities in forest floors under four tree species in coastal British Columbia. Soil Biology and Biochemistry 37, 1157–1167.
Grayston, S.J., Rennenberg, H., 2006. Assessing effects of forest management on microbial community structure in a central European beech forest. Canadian Journal of Forest Research 36, 2595–2604.
Guo, L.B., Gifford, R.M., 2002. Soil carbon stocks and land-use change: a meta analysis. Global Change Biology 8, 345–360.
Gutknecht, J.L.M., Henry, H.A.L., Balser, T.C., 2010. Inter-annual variation in soil extra-cellular enzyme activity in response to simulated global change and fire disturbance. Pedobiologia 53, 283–293.
Gutknecht, J.L.M., Field, C.B., Balser, T.C., 2012. Microbial communities and their responses to simulated global change fluctuate greatly over multiple years. Global Change Biology 18, 2256–2269.
Harris, J.A., 2003. Measurements of the soil microbial community for estimating the success of restoration. European Journal of Soil Science 54, 801–808.
Huang, Z.Q., Xu, Z.H., Chen, C.R., 2008. Interactions among soil organic matter fractions, microbial communities and nitrogen transformations in two hardwood plantations of subtropical Australia: effect of mulch. Applied Soil Ecology 40, 229–239.
Jangid, K., Williams, M.A., Franzluebbers, A.J., Blair, J.M., Coleman, D.C., Whitman, W.B., 2010. Development of soil microbial communities during tallgrass prairie restoration. Soil Biology and Biochemistry 42, 302–312.
Jangid, K., Williams, M.A., Franzluebbers, A.J., Schmidt, T.M., Coleman, D.C., Whitman, W.B., 2011. Land-use history has a stronger impact on soil microbial community than aboveground vegetation and soil properties. Soil Biology and Biochemistry 43, 2185–2193.
Jussy, J.H., Koerner, W., Dambrine, E., Dupouey, J.L., Benoit, M., 2002. Influence of former agricultural land use on net nitrate production in forest soils. European Journal of Soil Science 53, 367–374.
Keeler, B.L., Hobbie, S.E., Kellogg, L.E., 2009. Effects of long-term nitrogen addition on microbial enzyme activity in eight forested and grassland sites: implications for litter and soil organic matter decomposition. Ecosystems 12, 1–15.
Kluber, L.A., Tinnesand, K.M., Caldwell, B.A., Dunham, S.M., Yarwood, R.R., Bottomley, P.J., Myrold, D.D., 2010. Ectomycorrhizal mats alter forest soil biogeochemistry. Soil Biology and Biochemistry 42, 1607–1613.
Koerner, W., Dupouey, J.L., Dambrine, E., Benoit, M., 1997. Influence of past land-use on the vegetation and soils of present day forest in the Vosges mountains, France. Journal of Ecology 85, 351–358.
Kulmatiski, A., Beard, K.H., 2008. Decoupling plant-growth from land-use legacies in soil microbial communities. Soil Biology and Biochemistry 40, 1059–1068.
Kulmatiski, A., Beard, K.H., 2011. Long-term plant growth legacies overwhelm short-term plant growth effects on soil microbial community structure. Soil Biology and Biochemistry 43, 823–830.
Lauber, C.L., Strickland, M.S., Bradford, M.A., Fierer, N., 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biology and Biochemistry 40, 2407–2415.
Leadley, P., Periera, H.N., Alkemade, R., Fernandez-Manjarres, J.F., Proenca, V., Scharlemann, J.P.W., Walpole, M.J., 2010. Biodiversity Scenarios: Projections of 21st Century Change in Biodiversity and Associated Ecosystem Services. Technical Series 50, Montreal, 132 pp.
Lenton, T.M., Held, H., Kriegler, E., Hall, J.W., Lucht, W., Rahmstorf, S., Schnellhuber, H.J., 2008. Tipping elements in the Earth's climate system. Proceedings of the National Academy of Sciences of the United States of America (PNAS) 105, 1786–1793.
Mangan, S.A., Schnitzer, S.A., Herre, E.A., Mack, K.M., Valencia, M.C., Sanches, E.I., Bever, J.D., 2010. Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. Nature Letters 466, 752–756.
Müller-Westermeier, G., 1996. Klimadaten von Deutschland Zeitraum 1961-1990. Deutscher Wetterdienst, Offenbach.
Mummey, D.L., Stahl, P.D., Buyer, J.S., 2002. Microbial biomarkers as an indicator of ecosystem recovery following surface mine reclamation. Applied Soil Ecology 21, 251–259.
Nielsen, K.E., Dalsgaard, K., Nørnberg, P., 1987. Effects on soils of an oak invasion of a *Calluna* heath, Denmark, II. Changes in organic matter and cellulose decomposition. Geoderma 41, 97–106.
Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2011. Vegan: Community Ecology Package. R Package Version, pp. 117–118. http://CRANRprojectorg/package=vegan.
Peltzer, D.A., Wardle, D.A., Allison, V.J., Baisden, W.T., Bardgett, R.D., Chadwick, O.A., Condron, L.M., Parfitt, R.L., Porder, S., Richardson, S.J., Turner, B.L., Vitousek, P.M., Walker, J., Walker, L.R., 2010. Understanding ecosystem retrogression. Ecological Monographs 80, 509–529.
Potthast, K., Hamer, U., Makeschin, F., 2012. Land-use change in tropical mountain rainforest region of southern Ecuador affects soil microorganisms and nutrient cycling. Biogeochemistry 111, 151–167.
Pritsch, K., Raidl, S., Marksteiner, E., Blaschke, H., Agerer, R., Schloter, M., Hartmann, A., 2004. A rapid and highly sensitive method for measuring enzyme activities in single mycorrhizal tips using 4-methylumbelliferone-labelled fluorogenic substrates in a microplate system. Journal of Microbiological Methods 58, 233–241.
Ramirez, K.S., Craine, J.M., Fierer, N., 2012. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. Global Change Biology 18, 1918–1927.
Read, D.J., Perez-Moreno, J., 2003. Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance? New Phytologist 157, 475–492.
Rinkes, Z.L., Weintraub, M.N., De Forest, J.L., Moorhead, D.L., 2011. Microbial substrate preference and community dynamics during decomposition of *Acer saccharum*. Fungal Ecology 4, 396–407.
Rode, M.W., 1999. Influence of forest growth on former heathland on nutrient input and its consequences for nutrition and management of heath and forest. Forest Ecology and Management 114, 31–43.
Scheffer, M., Bascompte, J., Brock, W.A., Brovkin, V., Carpenter, S.R., Dakos, V., Held, H., van Nes, E.H., Rietkerk, M., Sugihara, G., 2009. Early-warning signals for critical transitions. Nature 461, 53–59.
Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial stress response physiology and its implications for ecosystem function. Ecology 88, 1386–1394.
Sinsabaugh, R., Carreiro, M.M., Repert, D.A., 2002. Allocation of extracellular enzymatic activity in relation to litter decomposition, N deposition, and mass loss. Biogeochemistry 60, 1–24.
Sparling, G.P., 1997. Soil microbial biomass, activity and nutrient cycling as indicators of soil health. In: Pankhurst, C.E., Doube, B.M., Gupta, V.V.S.R. (Eds.), Biological Indicators of Soil Health. CAB International, pp. 97–119.
Steubing, L., Fangmeier, A., 1992. Pflanzenökologisches Praktikum. Parey Verlag, Berlin.
Sun, B., Dong, Z.X., Zhang, X.X., Li, Y., Cao, H., Cui, Z.L., 2011. Rice to vegetables: short- versus long-term impact of land-use change on the indigenous soil microbial community. Microbial Ecology 62, 474–485.
Team RDC, 2012. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. http://wwwR-projectorg.
Verheyen, K., Bossuyt, B., Hermy, M., Tack, G., 1999. The land-use history (1278-1990) of a mixed hardwood forest in western Belgium and its relationship with chemical soil characteristics. Journal of Biogeography 26, 1115–1128.
Vestal, J., White, D., 1989. Lipid analysis in microbial ecology – quantitative approaches to the study of microbial communities. Bioscience 39, 535–541.
von Oheimb, G., Westphal, C., Härdtle, W., 2007. Diversity and spatio-temporal dynamics of dead wood in a temperate near-natural beech forest (*Fagus sylvatica*). European Journal of Forest Research 126, 359–370.
von Oheimb, G., Härdtle, W., Naumann, P.S., Westphal, C., Assmann, T., Meyer, H., 2008. Long-term effects of historical heathland farming on soil properties of forest ecosystems. Forest Ecology and Management 255, 1984–1993.
Wallenstein, M., Hall, E., 2012. A trait-based framework for predicting when and where microbial adaptation to climate change will affect ecosystem functioning. Biogeochemistry 109, 35–47.
Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., van der Putten, W.H., Wall, D.H., 2004a. Ecological linkages between aboveground and belowground biota. Science 304, 1629–1633.
Wardle, D.A., Walker, L.R., Bardgett, R.D., 2004b. Ecosystem properties and forest decline in contrasting long-term chrono-sequences. Science 305, 509–513.
Wakelin, S.A., Gregg, A.L., Simpson, R.J., Li, G.D., Riley, I.T., McKay, A.C., 2009. Pasture management clearly affects soil microbial community structure and N-cycling bacteria. Pedobiologia 52, 237–251.
Weintraub, M., Scott-Denton, L., Schmidt, S., Monson, R., 2007. The effects of tree rhizodeposition on soil exoenzyme activity, dissolved organic carbon, and nutrient availability in a subalpine forest ecosystem. Oecologia 154, 327–338.
Westphal, C., 2001. Theoretische Gedanken und beispielhafte Untersuchungen zur Naturnähe von Wäldern im Staatlichen Forstamt Sellhorn. Berichte Forschungszentrum Waldökosysteme A 174, 1–227.

Williams, M.A., 2007. Response of microbial communities to water stress in irrigated and drought-prone tallgrass prairie soils. Soil Biology and Biochemistry 39, 2750–2757.
Wong, M.-K., Gu, W., Ng, T.L., 1997. Sample preparation using microwave assisted digestion or extraction techniques. Analytical Science 13, 97–102.
Wu, Y.T., Gutknecht, J., Nadrowski, K., Geißler, C., Kühn, P., Scholten, T., Both, S., Erfmeier, A., Böhnke, M., Bruelheide, H., Wubet, T., Buscot, F., 2012. Relationships between soil microorganisms, plant communities and soil characteristics in Chinese subtropical forests. Ecosystems 15, 624–636.
Wurzburger, N., Hendrick, R.L., 2009. Plant litter chemistry and mycorrhizal roots promote a nitrogen feedback in temperate forest. Journal of Ecology 97, 528–536.
Zhang, L., Xu, Z.H., Patel, B.K.C., 2009. Culture-dependent and culture-independent microbial investigation of pine litters and soil in subtropical Australia. Journal of Soils and Sediments 9, 148–160.
Zuur, A.F., Ieno, E.N., Smith, G.M., 2007. Analysing Ecological Data. Springer, New York.