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Bracken fern (Pteridium aquilinum) is one of the most successful plant colonizers of soils in temperate regions; however, its effects on microbial community structure and activity and nutrient dynamics remain poorly understood. We studied whether colonization of forest soil by bracken fern modifies the structure and function of the soil microbial communities and considered the implications for ecosystem functioning. For this purpose, we analyzed microbial community structure (PLFAs) and activity (basal respiration, metabolic quotient), litter decomposition and nutrient dynamics (C, N and P) in monospecific oak (Quercus robur L.), eucalyptus (Eucalyptus globulus Labill.) and maritime pine forests (Pinus pinaster Aiton) colonized by bracken fern. Colonization of forest soil by bracken fern led to a reduction in differences in microbial community structure, as revealed by principal component and cluster analysis, although samples from oak forests were grouped separately. According to this, bracken litter decomposed to a greater extent than native tree litter in pine forest soils, whereas the opposite was found in oak forest soils. Such differences were not observed in eucalyptus forest soils. Colonization by bracken fern affected C mineralization, with no difference between the different types of forest; however, both N and P mineralization were higher in oak than in the other types of forest. In conclusion, colonization by bracken fern homogenizes soil microbial community structure. Differences in the decomposability of bracken litter in the different forest systems suggest a high degree of metabolic specialization of soil microorganisms. Thus, the soil microorganisms associated with bracken are continuously driven to decompose the bracken litter. In the long-term this will alter nutrient cycling, slowing decomposition and enhancing sequestering of nutrients by bracken ferns.

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Microbial community structure and nutrient dynamics in forest soils colonized by bracken fern (*Pteridium aquilinum*)

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Abstract

Bracken fern (*Pteridium aquilinum*) is one of the most successful plant colonizers of soils in temperate regions; however, its effects on microbial community structure and activity and nutrient dynamics remain poorly understood. We studied whether colonization of forest soil by bracken fern modifies the structure and function of the soil microbial communities and considered the implications for ecosystem functioning. For this purpose, we analyzed microbial community structure (PLFAs) and activity (basal respiration, metabolic quotient), litter decomposition and nutrient dynamics (C, N and P) in monospecific oak (*Quercus robur* L.), eucalyptus (*Eucalyptus globulus* Labill.) and maritime pine forests (*Pinus pinaster* Aiton) colonized by bracken fern. Colonization of forest soil by bracken fern led to a reduction in differences in microbial community structure, as revealed by principal component and cluster analysis, although samples from oak forests were grouped separately. According to this, bracken litter decomposed to a greater extent than native tree litter in pine forest soils, whereas the opposite was found in oak forest soils. Such differences were not observed in eucalyptus forest soils. Colonization by bracken fern affected C mineralization, with no difference between the different types of forest; however, both N and P mineralization were higher in oak than in the other types of forest. In conclusion, colonization by bracken fern homogenizes soil microbial community structure. Differences in the decomposability of bracken litter in the different forest systems suggest a high degree of metabolic specialization of soil microorganisms. Thus, the soil microorganisms associated with bracken are continuously driven to decompose the bracken litter. In the long-term this will alter nutrient cycling, slowing decomposition and enhancing sequestering of nutrients by bracken ferns.

Subjects: Soil Science, Microbiology, Ecology

Keywords: Litter decomposition, Metabolic quotient, Microbial activity, Microbial community structure, Nutrient cycling, Plant invasion

INTRODUCTION

Production of plant litter modifies the composition and/or the physiological capacities of soil microbial communities, which become specialized in decomposing particular types of litter (*Gholz et al., 2000; Ayres et al., 2009a; Ayres et al., 2009b*). It has been suggested that colonizing plant species will change the quantity or quality of litter, root exudates, release chemicals, display different nutrient acquisition/releasing patterns and alter soil structure via their rooting strategies (reviewed in *Wolfe & Klironomos, 2005*). As a consequence, microorganisms should respond with changes in their structure and function, and these effects should be more pronounced as differences in plant characteristics (litter, root exudates, etc) between local and incoming plant species increase (*Ayres et al., 2009a*). This process strongly depends on plant density and time since establishment of the new plant species, resulting in a short-term response mainly through physiological adaptations of soil microorganisms. In the long-term it will drive soil microbial communities to become more specialized (*Ayres et al., 2009a*).

The bracken fern (*Pteridium aquilinum* L. Kuhn) is one of the most widely dispersed and successful colonizing plant species worldwide (*Page, 1976; Harper, 1977*). This plant has some of the above-mentioned characteristics. First, it has a rhizome system that stores reserves and controls soil nutrient pools due to the uptake and storage, as nutrient recycling in bracken is very efficient and nutrients are sequestered even after senescence (*Lederle & Mroz, 1991; Werkman & Callaghan, 2001*). Second, it is highly productive, yielding a massive frond canopy that leads to accumulation of litter; and third, it releases several toxic chemicals to soil through the roots and fronds. These processes may strongly influence the microbial communities in soils colonized by bracken fern, thus altering biogeochemical cycles.

Here, we question how these processes affect forest soil microbial communities following colonization by bracken fern. Specifically, we tried to determine whether the modifications depend on the tree species present, so that forest soil microbial communities will be more resistant or more capable of coping with new litter types depending on forest litter quality.

In general, colonization of soils by bracken fern and the subsequent plant establishment lead to increased density and height of bracken fern over time (*Marrs & Watt, 2006*), and these traits govern the inputs of bracken litter to soil. Thus, we hypothesized that bracken colonization/establishment should modify the structure of soil microbial communities, homogenizing them in a way that is independent of their previous structure (due to dominant tree species). This change should affect ecosystem functioning, in this case measured as C, N and P mineralization and litter decomposition. This is important as the abundance and distribution of bracken fern, which are mainly limited by frost and waterlogging, are predicted to increase as a result of global climate change (*Marrs & Watt, 2006*). To test this hypothesis, we sampled soil in three types of forest, all heavily colonized by bracken fern: pine (*Pinus pinaster* Aiton), eucalyptus (*Eucalyptus globulus* Labill.) and oak (*Quercus robur* L.). We then analyzed the samples to determine the structure (PLFAs) and activity (basal respiration) of the microbial communities, as well as the concentrations of the major nutrients (C, N and P). We also included estimates of bracken fern height and density and tree age (estimated as trunk diameter) and density in our models to help clarify how bracken and tree species influence the structure of soil microbial communities. In addition, we conducted a litter decomposition experiment to test whether structural changes in the microbial communities of colonized soils were associated with functional changes, thereby favouring decomposition of bracken litter over native tree litter.

MATERIAL & METHODS

Site description

The study was conducted in late spring in southwest Galicia (42º12'N, 8º16'W), in an area (14000 ha) including pine, oak and eucalyptus forests at an elevation of 500 m above sea level. The area is within the Atlantic bioclimatic region. The distance between plots was no more than 8 km, thus ensuring that the different plots experience similar soil and macroclimate conditions. We randomly selected four monospecific forests (plots) for each of the three tree species (pine, oak and eucalyptus). All plots were densely colonized by bracken fern. The litter layer in the plots comprised a mixture of tree and bracken fern litter, although tree litter was the most abundant in all cases. In accordance with this, we randomly established four sampling plots (each 1 m^2) within each plot, at least 2 m from the closest tree. We then obtained five composite soil samples per plot for analysis of chemical and microbiological parameters. In each plot, we measured the height and density of bracken ferns and the trunk diameter (at breast height) of the three trees nearest to the plot, and we used the n-tree protocol (*Lynch & Rusydi, 1999*) to calculate the tree density. Tree density and trunk diameter are surrogate measures for respectively litter input and forest age, and bracken fern density and height indicate colonization success. Thus, tree density ($F_{2,9}$=2.43, P=0.14) and age, estimated as trunk diameter ($F_{2,9}$=3.02, P=0.10), did not differ between the three forest types. The density of bracken fern was also independent of tree species ($F_{2,9}$=3.38, P=0.08) and was not affected by tree density or age. Height of bracken ferns was also strongly influenced by tree species ($F_{2,9}$=14.98, P=0.001), with higher fronds in pine (128±7 cm) than in oak plots (79±8 cm), and fronds in the eucalyptus plots being of intermediate height (115±10 cm). Furthermore, bracken was shorter in oak forest plots

with mature trees and it was taller in pine and eucalyptus plots (interaction tree age x tree species, $F_{2,29}$=8.81, *P*=0.001).

Analytical methods

The moisture content of the soil samples was determined after drying at 105°C for 24 h, and the organic matter content was determined after heating at 550°C for 4 h. The pH was measured in a suspension of the samples in distilled water, at a sample to extractant ratio of 1:20 (weight/volume). Inorganic N ($N\text{-}NH_4^+$ and $N\text{-}NO_3^-$) was determined in 0.5M K_2SO_4 extracts (1:5 weight/volume) by a modified indophenol blue technique (*Sims et al., 1995*), and the absorbance was read in a microplate reader (Bio-Rad Model 550). Total extractable N was determined in 0.5M K_2SO_4 extracts after oxidation with $K_2S_2O_8$, as described by *Cabrera & Beare (1993)*. The dissolved organic nitrogen (DON) content was calculated as (total extractable N) - ($N\text{-}NH_4^+$ and $N\text{-}NO_3^-$). Dissolved organic carbon (DOC) was determined colorimetrically after moist digestion ($K_2Cr_2O_7$ and H_2SO_4) of aliquots of 0.5 M K_2SO_4 extracts of the samples. Phosphate was extracted from soil samples (2 g dw) with acetic acid (2.5%), filtered and the absorbance was read at 700 nm after the addition of ammonium molybdate (0.1M) and tin chloride (*Allen et al., 1986*).

Microbial communities were assessed by phospholipid fatty acid (PLFA) analysis. Total lipids were extracted from soil samples (2 g dry weight) with methanol and chloroform (1:2 v:v) and the mixture was filtered and evaporated under a stream of N_2 gas. The total lipid extract was then dissolved with chloroform (3 x 1 ml). Lipids were separated into neutral, glycol- and phospholipids on silicic acid columns (Strata SI-1 Silica (55 µm, 70 A), 500 mg/6 ml) with chloroform, acetone and methanol. The fraction containing phospholipids was evaporated under a stream of N_2 and redissolved in 500 µl of methyl-*tert*-butyl ether. An aliquot of 100µL of this solution was placed in a 1.5 mL

vial with 50 µL of the derivatizating agent (trimethylsulfonium hydroxide, TMSH), vortexed for 30 s and allowed to react for 30 min; nonadecanoic acid methyl ester (10 µL) was added as an internal standard. The chromatographic conditions are described elsewhere. To identify and quantify the fatty acids, retention times and mass spectra were compared with those obtained for known standard mixtures or pure PLFAs (*Gómez-Brandón et al., 2008; Aira et al., 2009*).

The PLFAs used as biomarkers have previously been defined (*Frostegård & Bååth, 1996; Zelles, 1999; Bååth, 2003*). Total microbial biomass was determined as the sum of all extracted PLFAs expressed as µg g^{-1} dry weight. Abundances of the different microbial groups (bacteria and fungi) were determined by the abundance of specific biomarkers commonly used for these groups. Bacterial biomass was determined as the sum of PLFAs considered to be predominantly of bacterial origin (i14:0, i15:0, a15:0, i16:0, i17:0 and a17:0c16:1ω7c, cy17:0, c17:1ω8, 18:1ω7c and cy19:0) (*Frostegård & Bååth, 1996*). The c18:2ω6c PLFAs were used as biomarkers for fungal biomass (*Frostegård & Bååth, 1996; Bååth, 2003*).

The basal and substrate induced respiration (SIR) of microbial communities in soil samples were determined. Briefly, the samples (20g fresh weight) were placed in glass jars, sealed and incubated at room temperature for 6 hours. Five ml of a glucose solution (8 mg ml^{-1}) was added to samples for SIR. The metabolic quotient (qCO_2) was also determined as the ratio of basal respiration to SIR (*Dearden & Wardle, 2007*) as a relative measure of carbon use efficiency and the extent of substrate limitation to soil microorganisms (*Anderson & Domsch, 1985; Wardle & Ghani ,1995*).

The decomposability of forest and bracken litter was determined using a standardized laboratory bioassay (*Wardle et al., 1998*). Briefly, 1 g (dry weight) of each litter type (bracken and forest) was placed on a nylon mesh (1mm diameter) and incubated in

individual Petri dishes (9 cm diameter) filled to 2/3 of capacity with the corresponding forest soil, and the soil moisture was adjusted to 25%. The Petri dishes were sealed with tape, to minimize water loss, and incubated at 20ºC for 90 days. In addition, some Petri dishes were prepared with mixtures of litter (bracken and tree) to assess any interactive effects of bracken and tree litter from each plot when mixed together. These Petri dishes were prepared as before, except that 0.5 g of each type of litter was added to each dish. After 90 and 180 days, the remaining litter was recovered, washed and dried (75ºC). The litter decomposition rate was determined as the percentage of mass lost during incubation.

Statistical analysis

Variables were transformed in order to fulfil assumptions of normality and homoscedasticity. Thus, tree and fern density, basal respiration, metabolic coefficient, nitrate and phosphate contents were log transformed. Total PLFA, bacterial, fungal, Gram-negative PLFAs and ammonium content were transformed using the box.cox function in MASS library (*Venables & Ripley, 2002*). Data were analyzed by fitting linear mixed models with the lme function in the nlme library (*Pinheiro et al., 2009*), in which tree species was included as a fixed factor, and bracken height and density, trunk diameter and tree density were included as covariates. For litter decomposition experiments, tree species and litter type (tree and bracken litter and the litter mixture) were included as fixed factors. Plot identity was nested into plots and included as a random factor to remove pseudoreplication (*Crawley, 2007*). Model fit was analyzed by graphical inspection of the residuals and by the linear relationship between the response variable and fitted values and error distribution (normal) in the four plots (*Crawley, 2007*). For comparison of abiotic and biotic soil variables between the three tree species (Tukey test), we used the glht function in the multcomp library (*Hothorn et al., 2008*).

In order to analyze the underlying effect of bracken colonization on soil microbial community structure, PLFA data were subjected to principal component analysis with the dudi.pca function of the ade4 library (*Dray & Dufour, 2007*). Cluster analysis was also used to estimate relationships between samples on the basis of similarities in the PLFA profiles. The Euclidean distance method was used to determine the distances in space, and the Ward method was used to add samples to clusters. The pvclust function of pvclust library was used to assess the uncertainty in hierarchical cluster analysis via multiscale boostrap resampling (10000 bootstrap replications). Thus, for each cluster, two types of *p*-values were calculated: approximately unbiased (AU) and bootstrap values (BP), the first of which is a better approximation of the unbiased *p*-value (*Shimodaira, 2002, 2004*). Values of AU and BP higher than 95 significantly ($P<0.05$) support the cluster. All analyses were conducted in the R environment.

RESULTS

Colonization of forest soils by bracken fern strongly modified the microbial community structure, decreasing the differences between the soils inhabited by three tree species (Fig. 1; PC1 and PC2 explained 57 and 15% of variance respectively). Furthermore, microbial communities of soils grouped into two main clusters both of which were significantly supported (AU *p*-value=97): one comprised two plots of oaks and another comprising the remaining samples (Fig. 2). This large cluster encompassed two main clusters, one comprising samples from eucalyptus and pine forests (AU *p*-value=97) and another comprising samples from two other oak forests (AU *p*-value=95). This suggests that bracken may play an important role in structuring microbial communities by reducing differences in their structure.

Despite the structuring effect, bracken did not alter soil microbial biomass contents, which remained largely dependent on tree species ($F_{2,9}=10.68$, $P=0.004$) with up to two

times more microbial biomass in oak forests than in pine and eucalyptus forests (Fig. 3a); interestingly, bracken density and height did not affect microbial biomass. Similarly, bacterial ($F_{2,9}$=11.27, P=0.003) and fungal PLFAs ($F_{2,9}$=6.68, P=0.016) also differed significantly between the three type of forests. Thus, the bacterial PLFA contents were 2.7 times higher in oak forests than in eucalyptus and pine forests (Fig. 3c). The fungal PLFA contents were higher in oak (2.7 times) and eucalyptus forest (3.6) than in pine forest (Fig. 3b). In contrast to the lack of effect on microbial biomass, bracken colonization promoted similar levels of microbial activity ($F_{2,9}$=2.14, P=0.17). However, it did not determine the proportion of active microorganisms, measured as substrate induced respiration, which peaked in oak forest and was up to 1.4 and 2.1 times higher than in pine and eucalyptus forest respectively ($F_{2,9}$=7.11 P=0.014; Fig. 3d). Regardless of this, the presence of bracken reduced microbial efficiency in a similar way in all three types of forests ($F_{2,9}$=3.49, P=0.075). Furthermore, none of the microbial parameters analyzed depended on tree density, tree age, bracken density or bracken height.

Bracken colonization modified soil moisture content, which decreased in the order oak to eucalyptus to pine soils ($F_{2,9}$=9.16, P=0.006; Table 1). Soil organic matter content differed between forest ($F_{2,9}$=8.53, P=0.008; Table 1), with bracken density increasing organic matter content in eucalyptus and pine forests, and decreasing it in oak forests (interaction tree species x bracken density, $F_{2,18}$=3.97, P=0.037). Bracken presence slightly altered N pools; thus, although the ammonium content varied between forests ($F_{2,9}$=5.22, P=0.031; Table 1), it decreased with the height of bracken ($F_{1,32}$=, P=0.046). Furthermore, the presence of bracken eliminated differences in soil nitrate content of three forests soils ($F_{2,9}$=0.58, P=0.39; Table 1), although the difference increased significantly with tree age ($F_{1,32}$=26.78, P<0.0001). However, the presence of bracken

did not determine dissolved organic N, the contents of which differed between forest soils ($F_{2,9}$=6.48, P=0.018 Table 1). Nonetheless, the presence of bracken determined dissolved organic C content of soils, which did not differ between forest soils ($F_{2,9}$=1.01, P=0.40; Table 1). The soil phosphate content differed between forest soils ($F_{2,9}$=28.79 P=0.0001; Table 1) and increased significantly with tree age ($F_{1,18}$=13.52, P=0.001). Nevertheless, in oak and pine forest soils the P content increased with bracken density, whereas the P content decreased with bracken density in eucalyptus soils (interaction tree species x bracken density, $F_{2,18}$=6.41, P=0.007).

In accordance with differences in microbial community structure, the rates of litter decomposition strongly depended on litter type and tree species (interaction forest x litter, $F_{4,90}$ = 14.31, P < 0.0001; Fig. 4). Thus, in oak forest soils, oak litter decomposed to a greater extent (1.4 times) than the bracken and litter mixture, whereas bracken litter decomposed to a greater extent (1.8 times) than pine litter and litter mixture in pine forest soils (Fig. 4). The decomposability of bracken and tree litter did not differ in eucalyptus forest soils (Fig. 4). After incubation of litter for 180 days, decomposition was on average about 3 points higher but with the same pattern across treatments (interaction forest x litter, $F_{4,90}$ = 10.91, P < 0.0001; data not shown). We did not find any effect of bracken or forest covariates on litter decomposition.

DISCUSSION

The success of bracken fern colonization was highly dependent on forest type, with shorter fronds in oak forest than in pine and eucalyptus forest. Surprisingly, tree age did not have a clear effect on the brackens, as frond height increased with age of pine and eucalyptus plots and decreased with age of oak plots. These data reveal contrasting patterns of colonization, which should decrease (oak) or intensify (pine and eucalyptus) the effects of bracken on the three forests soil microbial communities. This occurred

despite the lack of differences in tree density and age among the three species, which suggests another type of control that trees may exert on brackens. It has been suggested that bracken proliferation in woodlands is controlled by a combination of reduced light and moisture conditions (*Marrs & Watt, 2006*). Although we did not measure the light conditions, brackens responded to moisture and grew taller in dry (eucalyptus) than in moist (oak) soils.

The presence of bracken also modified the structure of forest soil microbial communities independently of tree species, as revealed by overlapping of inertia ellipses in PCA and grouping of data in cluster analysis. However, the structure of microbial communities was slightly different in oak forest than in pine and eucalyptus forest, as may be expected by the poor bracken performance (bracken height and density) in these forests. Furthermore, the presence of bracken did not eliminate differences in overall microbial biomass, or bacterial and fungal biomass, which were both higher in oak than in pine and eucalyptus forest. Our results are consistent with those *of Kourtev et al. (2002, 2003)*, who found that plant invasion differentiated soil microbial community structure. Our data contrast with the clear differentiation of microbial communities in several types of forests, as indicated by PLFA profiles and PCA analysis, with the explained variance (first two principal components) ranging between 40 and 77% (*Waldrop et al., 2000, 2004; Priha et al., 2001; Myers et al., 2001; Kourtev et al., 2003; Gallo et al., 2004*), thus supporting our hypothesis of microbial community homogenization due to bracken activity. However, despite evident differences in microbial biomass, we did not observe any variation in microbial activity or the metabolic quotient, which indicates that resource use efficiency was not affected. Moreover, the low values of metabolic quotient (< 1 in all samples) indicate that microorganisms were in an “energy-saving” state. This may be due to low digestibility

of bracken litter, as the rate of decay is slower than the renewal rate, with complete decomposition taking as long as eleven years (*Frankland,1966a,b; 1976*). Furthermore, this may be the main reason for the strong modifications in microbial community structure in the stands with heavier bracken presence. *Waldrop & Firestone (2004)* also found that different plant communities did not modify the microbial communities that decompose readily decomposable substrates but also those that decompose the more recalcitrant substrates.

We found that bracken modified both the microbial community structure and its decomposition capacity, which differed between forest types. Thus, microbial communities in oak forest soils were better at decomposing oak litter than bracken litter, whereas those in pine forest soils were better at decomposing the bracken litter, although differences were not observed in eucalyptus forest soils. Moreover, differences in litter decomposability in oak forest soils were not as marked as those observed in pine forest soils, possibly indicating two extremes of a process of microbial adaptation to bracken litter. Interestingly, mixtures of bracken and tree litter always decomposed at the same rate as the most recalcitrant litter (bracken or tree), indicating that the recalcitrant litter may determine the optimal functioning of microorganisms due to lack of adaptation to their chemical composition (*Hättenschwiler et al., 2005*). This suggests a high degree of metabolic specialization of soil microorganisms, so that those associated with bracken are continuously driven to decompose bracken litter.

As a consequence of the adaptation of microbial communities to the specific processes in the bracken life cycle, ecosystem functioning was also altered, and slight differences in some nutrient pools (ammonium, dissolved organic nitrogen and phosphate contents) were observed. However, no changes were observed in the rate of C mineralization (as indicated by basal respiration and dissolved organic contents). In accordance with

microbial parameters, which showed that microbial communities in oak soil differ from those in other forest soils, the N and P mineralization rates were higher in oak than in pine and eucalyptus soils. These data suggest that nutrient mineralization is still determined by tree species, although bracken colonization began to be important, exerting different effects depending on nutrient and tree species.

CONCLUSIONS

In conclusion, colonization of soil by bracken fern and subsequent plant establishment altered the microbial community of soils, driving it to a similar structure and functioning. In the long-term this will alter nutrient cycling, slowing decomposition and even sequestering nutrients. Moreover, homogenization of the soil microbial community may have profound effects on ecosystem functioning and ecosystem resilience to disturbance (*McKinney and Lockwood, 1999; Olden et al., 2004; Rodrigues et al., 2013*).

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Competing interest

The authors declare there are no competing interest

Author contributions

Manuel Aira designed the experiment, performed the experiments, analyzed the data, wrote the paper, reviewed drafts of the paper

Andrea Tato performed the experiments, analyzed the data

Jorge Domínguez designed the experiment, performed the experiments, wrote the paper, reviewed drafts of the paper

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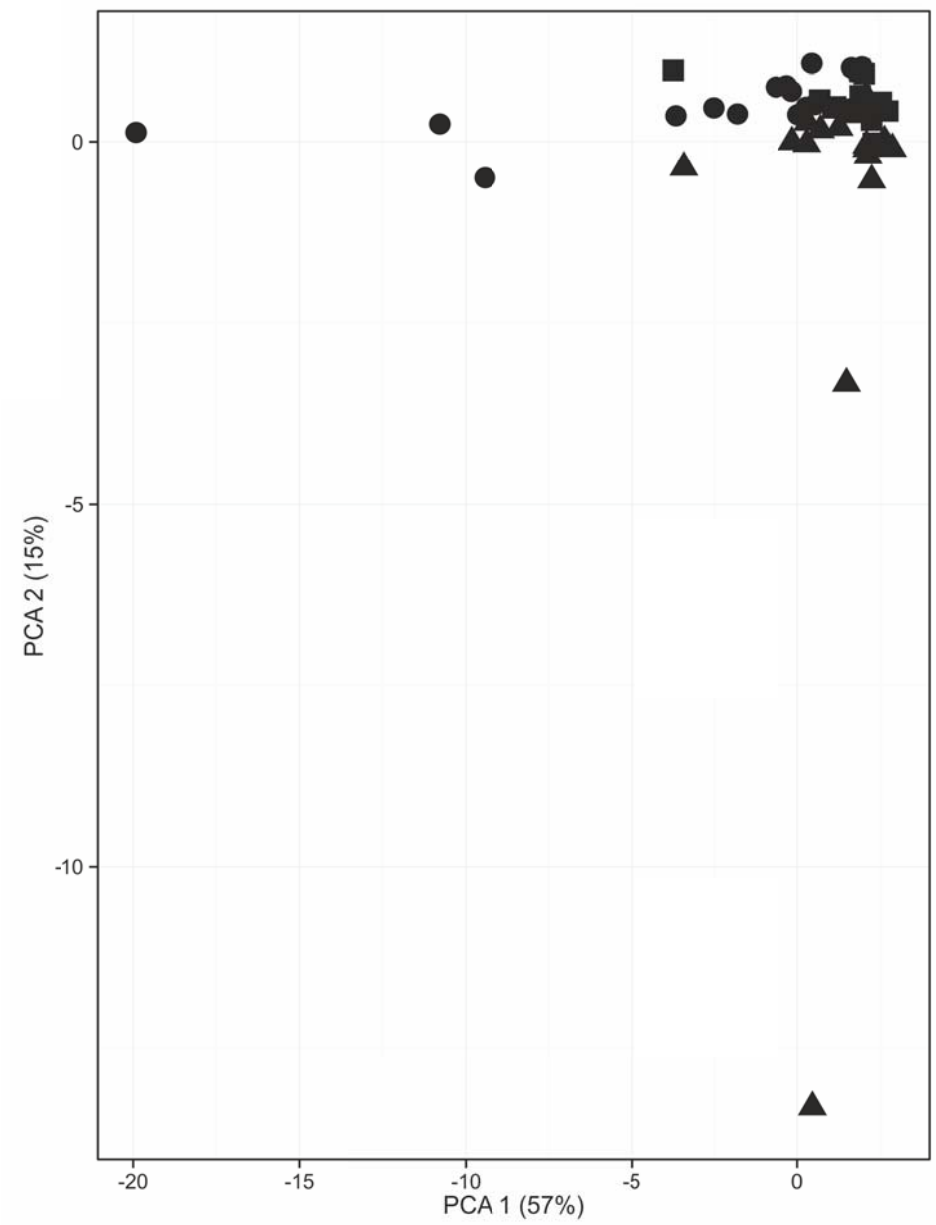

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519 Figure 1. Principal component analysis of the PLFA matrix obtained from samples of

520 oak, pine and eucalyptus forests (circle, square and triangle symbols respectively)

521 colonized by bracken fern.

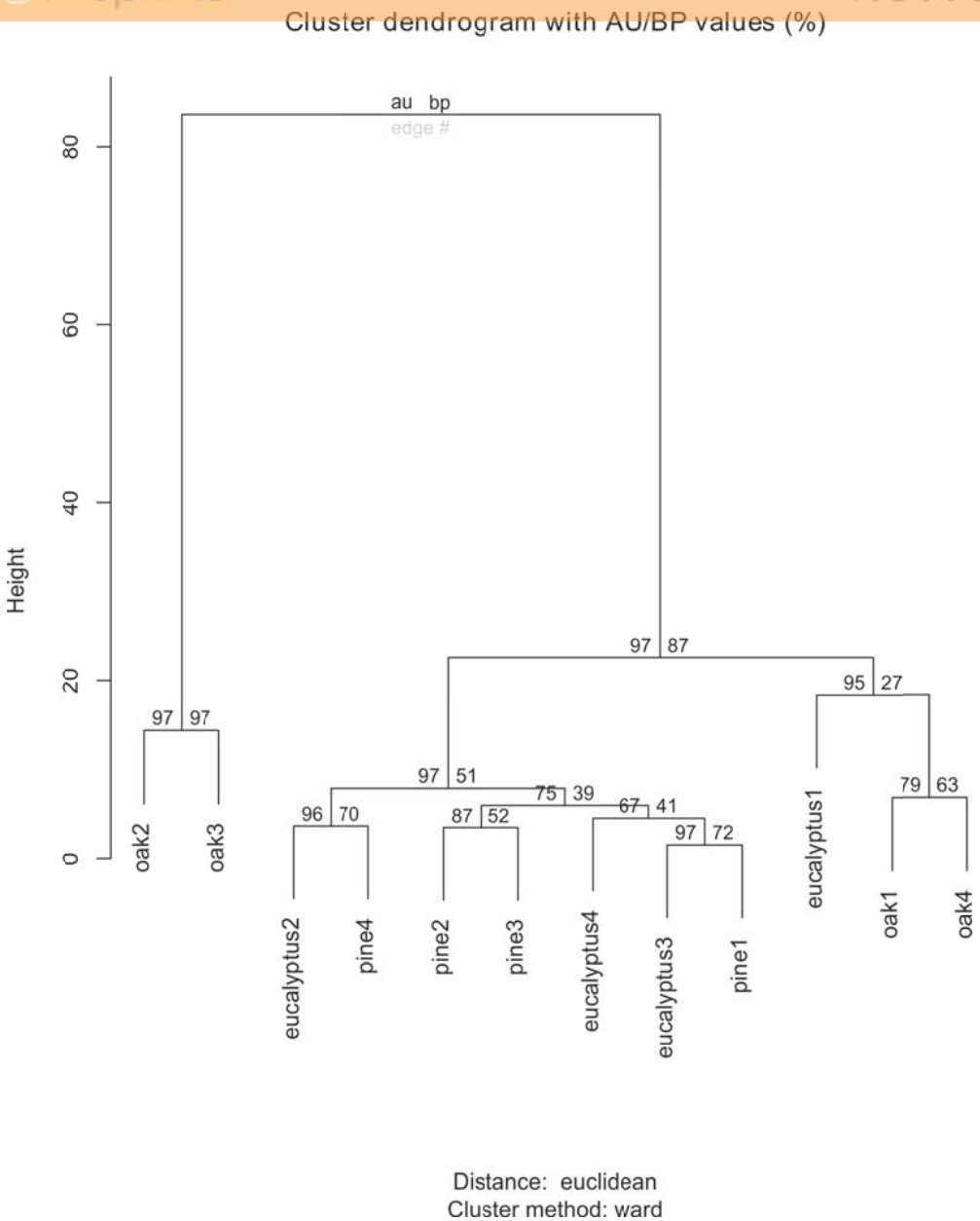
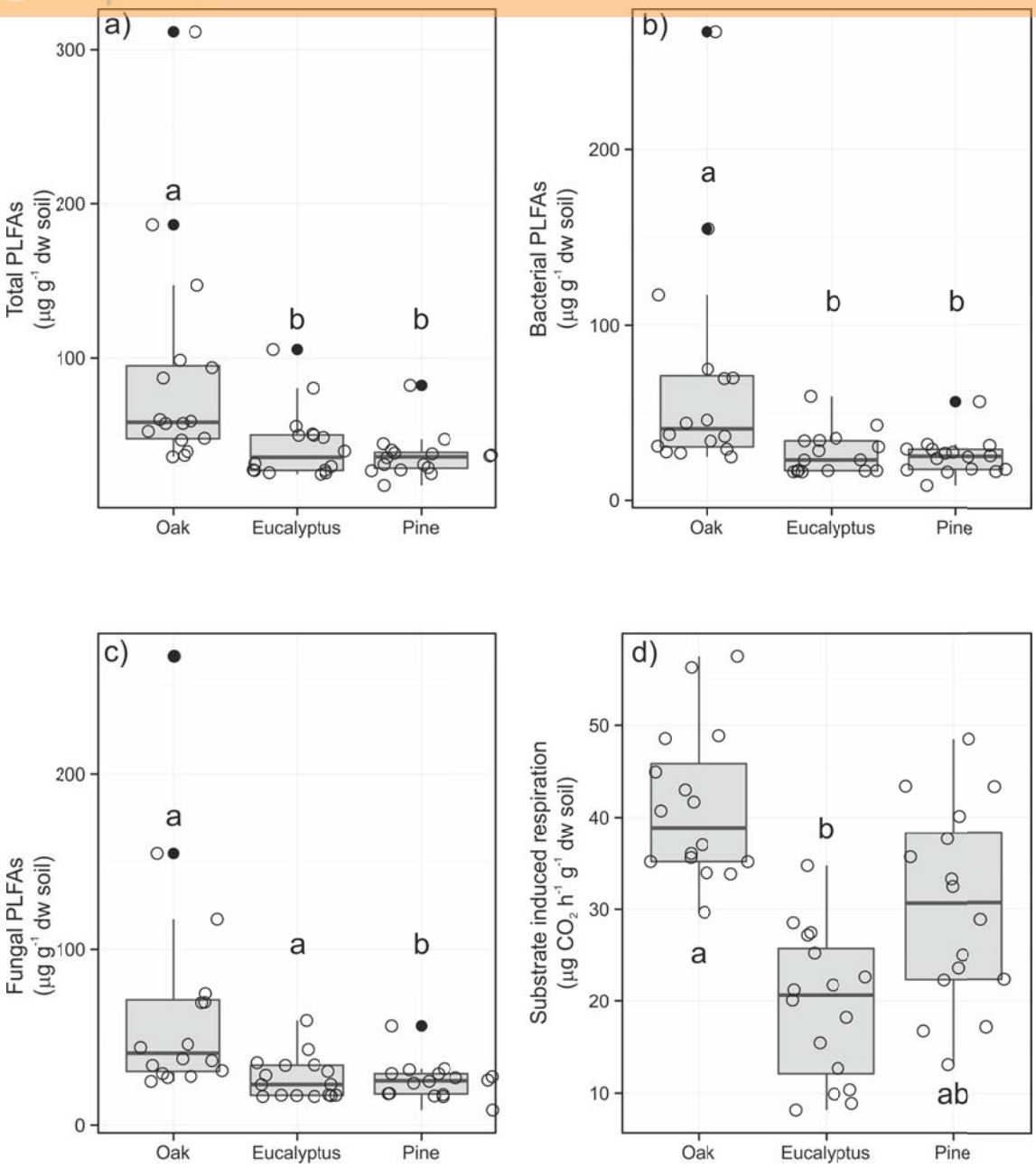


Figure 2. Cluster analysis of PLFA profile of three forest samples. Clusters were determined by the Ward method and by Euclidean distance. Numbers at nodes are approximately unbiased (au) and bootstrap probability *p*-values.



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529 Figure 3. Microbial biomass and activity in soils colonized by bracken fern. (a)
530 Microbial biomass, (b) fungal biomass, (c) bacterial biomass and (d) substrate induced
531 respiration. White and black dots represent sample values and outliers respectively.
532 Different letters indicate significant differences based on multiple comparisons (Tukey
533 HSD test).

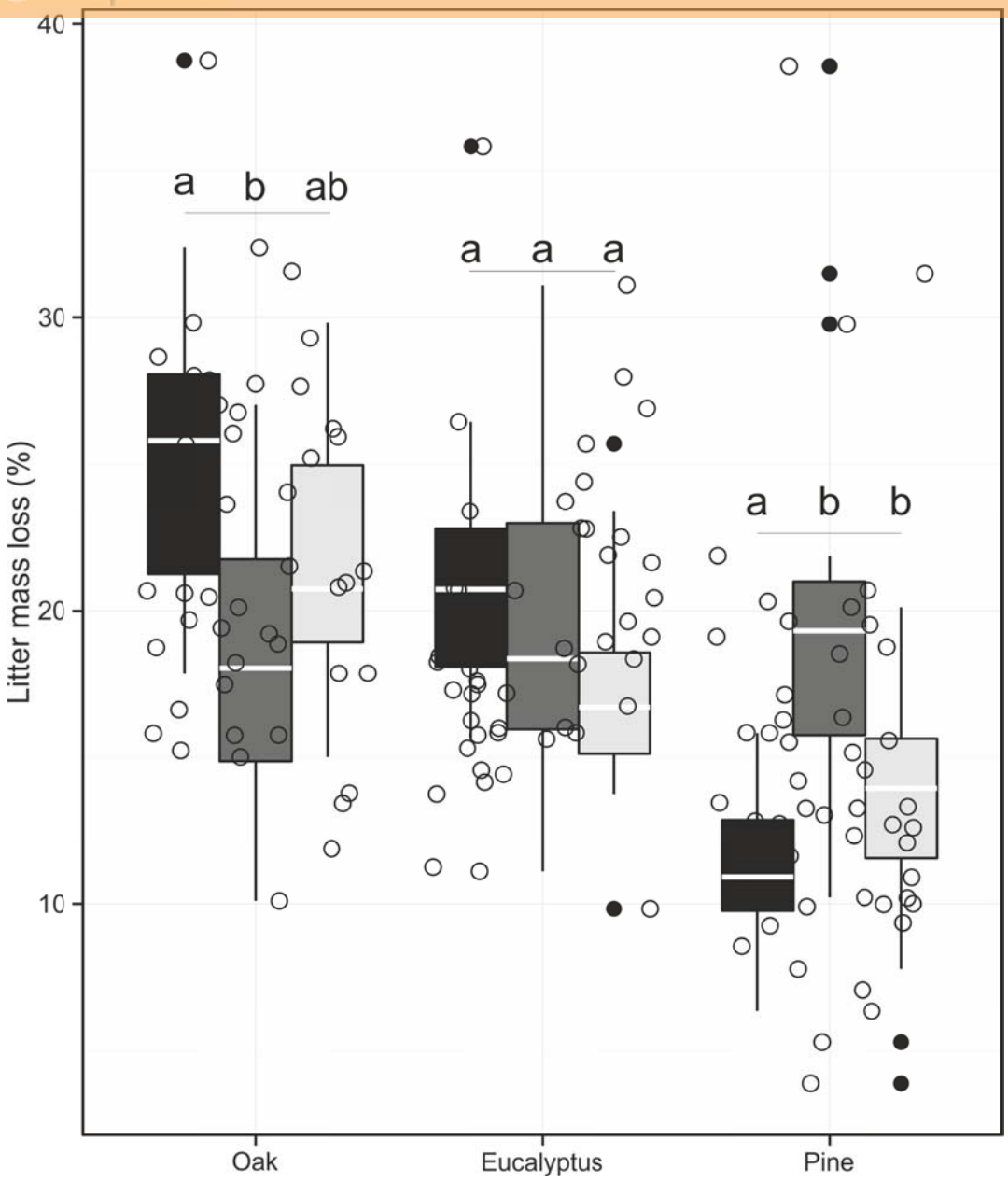

Figure 4. Litter decomposition of forest and bracken litter and mixtures of these (black, dark grey and light grey respectively) in eucalyptus, pine and oak soil colonized by bracken fern (*Pteridium aquilinum*). White and black dots represent sample values and outliers respectively. Different letters indicate significant differences based on multiple comparisons within each forest soil (Tukey HSD test).

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538 Table 1. Chemical characteristics of oak (*Quercus robur*), pine (*Pinus pinaster*) and
539 eucalyptus (*Eucalyptus globulus*) soils colonized by bracken fern (*Pteridium*
540 *aquilinum*). Different letters indicate significant differences based on multiple
541 comparisons (Holm test). Values are means ± standard error.

	Oak	Pine	Eucalyptus
Moisture content (%)	24±1a	20±1ab	15±1b
Organic matter content (%)	24±1a	23±1ab	17±1b
$N\text{-}NH_4^+$ (μg g^{-1} dw)	15±2a	14±1a	9±1b
$N\text{-}NO_3^-$ (μg g^{-1} dw)	11±1	12±1	11±1
Dissolved organic N (μg g^{-1} dw)	101±5a	81±5b	73±5b
Dissolved organic C (μg g^{-1} dw)	1090±110	925±100	890±60
Phosphate content (μg g^{-1} dw)	41±5a	22±2b	18±2b

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