Biotic and Abiotic Constraints on the Decomposition of *Fagus sylvatica* Leaf Litter Along an Altitudinal Gradient in Contrasting Land-Use Types

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ABSTRACT

Climate change can affect the process of carbon cycling and leaf litter decomposition in multiple ways, both directly and indirectly, though the strength and direction of this relationship is often context dependent. In this experiment, we followed decomposition of a standard litter type—senescent leaves of *Fagus sylvatica* collected from a single location—along a 1000 m altitudinal gradient of four sites over 2.5 years. To control the edaphic conditions, we transplanted intact turf mesocosms from three different land-use types [densely wooded, sparsely wooded, and unwooded (UW) pastures] from the highest altitude site into UW pastures along the altitudinal gradient from the moist, cool high-elevation site to the dry, warm low-elevation site, using shade cloth to mimic the light conditions in the original habitats. Decomposition in the drier UW pasture mesocosms increased with altitude, likely because of higher moisture at the highest sites. Decomposition in the more mesic mesocosms from sparsely and densely wooded sites was insensitive to altitude, suggesting an overriding moisture, rather than temperature, constraint on decomposition across these sites. The functional composition of decomposer microbial communities (fungal/bacterial ratio) was similarly insensitive to altitude. Our findings bring substantial evidence for the controlling role of soil moisture on litter decomposition, as well as for the indirect effects of climate through changes in the decomposer community.

Key words: beech; climate change; C/N ratio; litter bag; litter decay rate (K); fungal/bacterial ratio; JURA mountains; pasture-woodlands; PLFA; transplantation.

INTRODUCTION

Litter and soil organic matter decomposition represent one of the major carbon fluxes from terrestrial ecosystems (Houghton 2007). Environmental conditions directly affect the rates of litter decomposition (Chapin and others 2002) such that in cold environments, such as mountain regions, ecosystems contain most of their carbon in the soil organic matter due to physiological constraints on the decomposer community (Körner 2003). In such cold biomes, temperature exerts a hierarchical control on litter decomposition, however, soil moisture modulates its direct positive effect, or even overrides it, based on regional precipitation and evapotranspiration regimes (Aerts 2006). In milder climates, litter quality is much more important for the rate of decomposition, especially during the initial stage of mass loss when labile substances are released (Couteaux and others 1995), but also during the subsequent degradation of recalcitrant material by filtering the composition of microbial communities (Bray and others 2012). This demonstrates how climate can also have indirect effects on decomposition mediated by a change in vegetation or in its litter quality (Cornelissen and others 2007; Cornwell and others 2008), but also by vegetation's feedback on soil microclimate (Körner 2003). Concomitant climate-driven changes in the soil microbial communities also affect the carbon cycle (Bardgett and others 2013) and litter decomposition in particular (Allison and others 2013), thus exerting another indirect climate effect.

In a changing climate (Solomon and others 2007), where mountain systems are said to experience a disproportionately higher increase in temperature (Nogues-Bravo and others 2007), the coupling between atmospheric and soil climatic conditions (Seneviratne and others 2006) can induce positive climate change feedbacks with far reaching implications for future terrestrial carbon cycling and litter decomposition. Reports on the confounding effect of soil moisture in climate warming experiments on litter decomposition come from a range of studies in both high latitude (Robinson 2002) and high altitude (Gavazov 2010) regions. These studies suggest a link to a documented strong moisture limitation of underlying metabolic processes in the soil (Steinweg and others 2012). As soil moisture is an essential control of C turnover and co-varies with changing temperature (Mueller and Seneviratne 2012), it is important to assess the impact of these environmental parameters on litter decomposition in concert.

Altitudinal gradient studies represent a natural approach to evaluate the impact of climatic parameters on ecological processes (Körner 2007). In particular, transplantation studies along climatic gradients present great potential for integrating ecosystem-scale climate change manipulations with natural environmental variation along substantial temporal and spatial scales (Beier and others 2012). They grant the possibility of mapping the response of an ecological process to a large range of variation in a given environmental parameter of interest (that is, temperature, precipitation, and so on), while controlling for the effect of others (that is, substrate, edaphic factors), establishing a causal relationship through a statistical regression approach (Cottingham and others 2005).

In this study we present data on decomposition rates of a standard litter type—senesced leaves of European beech *Fagus sylvatica*—along an altitudinal gradient of approximately 1000 m spanning from the subalpine (wet and cold) to the colline (dry and warm) vegetation zones in the Swiss Jura Mountains (Figure 1A). To standardize the edaphic conditions along the gradient, prior to litter bag incubation in the field, four common gardens were set up at distinct elevations, in which we transplanted intact turf mesocosms from three different land-use types (see Table 1) originating from an area of mosaic pasture-woodlands at the crest of the Jura Mountains. Alongside mass loss and chemical composition of the decaying litter, we monitored the temporal dynamics of the decomposer microbial community. Based on the climatic gradient used, we hypothesized that beech litter decomposition rates will be most rapid within an optimum climatic range at mid-altitude and experience soil moisture limitation at lower altitude and temperature limitation at higher altitude (Figure 1B). Distinct trajectories were expected for the three land-use types, such that at the top of the gradient unwooded (UW) pastures, characterized by rich grassland communities, would provide better conditions for litter decomposition in terms of a warmer microclimate, and a microbial community adapted to a faster soil organic matter cycle. However, at the bottom of the gradient, the pattern will be reversed since wooded pastures will experience less soil moisture limitation due to their canopy shading.

MATERIALS AND METHODS

Experimental Design and Litter Bag Preparation

The study was conducted within an existing turf-transplantation experiment at four distinct
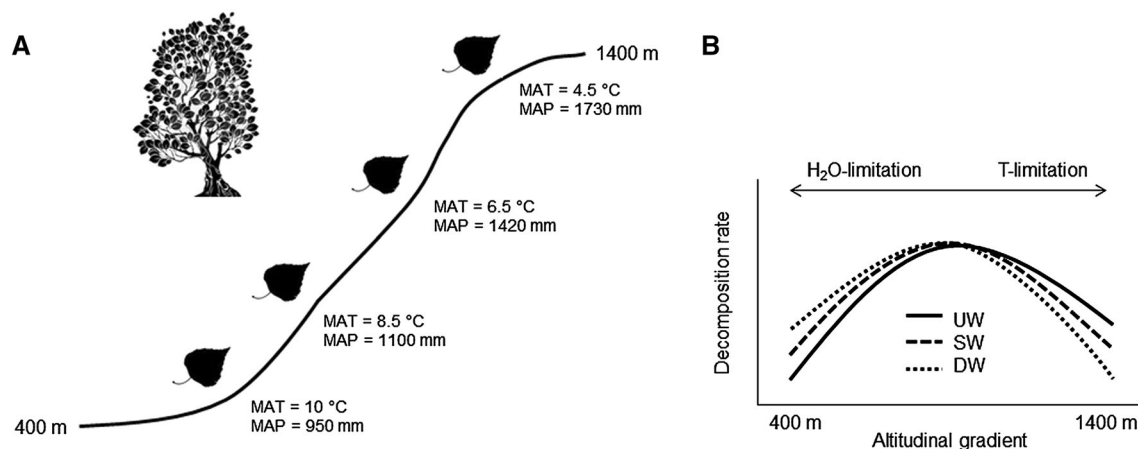
Figure 1. **A** Schematic representation of the altitudinal gradient used in this study and the corresponding climate experienced by litter bags with *Fagus sylvatica* leaves incubated on the surface of transplanted mesocosms. **B** A conceptual chart demonstrating the hypothetical controlling role of soil moisture and soil temperature on the litter decomposition process in each of the three studied land-use types: unwooded pasture (UW), sparsely wooded pasture (SW), and densely wooded pasture (DW).

Table 1. Biogeochemical Characteristics of the Topsoil Layer (0 to −10 cm) at the Control Site at 1350 m a.s.l

Land use type[1]	UW	SW	DW
Bulk density (g/cm^3)	0.57 ± 0.01	0.54 ± 0.04	0.55 ± 0.02
pH in H_2O	5.88 ± 0.06	5.47 ± 0.15	5.85 ± 0.08
SOM (g/g dry soil)	0.20 ± 0.01	0.18 ± 0.01	0.20 ± 0.01
C/N ratio	15.90 ± 0.57	18.83 ± 0.62	20.25 ± 0.42
Soil PLFA fungal/bacterial ratio	0.200	0.217	0.350
Mean annual soil temperature (°C)	6.58 ± 0.15	6.07 ± 0.16	5.70 ± 0.11
Mean annual soil moisture (%)	42.3 ± 3.6	37.6 ± 2.9	28.8 ± 2.5

[1]*Land-use type codes, where UW refers to unwooded pasture with 0% canopy shading, SW refers to sparsely wooded pasture with 40% canopy shading, and DW refers to densely wooded pasture with 80% canopy shading.*

altitudes simulating effects of climate change on pasture woodlands in the Swiss Jura Mountains (see Gavazov and others 2014). We used this gradient as a proxy for different in situ climatic conditions as represented in Figure 1A. Briefly, at each given altitude: 1350 m a.s.l. (Combe des Amburnex, N 46°54′, E 6°23′), 1010 m a.s.l. (Saint-George, N 46°52′, E 6°26′), 570 m a.s.l., (Arboretum d'Aubonne, N 46°51′, E 6°37′), and 395 m a.s.l. (Les Bois Chamblard, N 46°47′, E 6°41′), a common garden was set up within an open field with individual mesocosms. Those were made of rectangular PVC boxes 60 × 80 cm and 35 cm in height, fitted with a drainage system (that is, a lysimeter), and dug down to soil surface level, thus preventing lateral heat exchange with the atmosphere. The intact turf monoliths for each mesocosm were translocated from the donor site at Combe des Amburnex (N

46°55′, E 6°24′)—a mosaic area of subalpine pasture-woodlands situated along the crest of the Swiss Jura Mountains, at circa 1350 m a.s.l. The landscape there is dominated by Norway spruce *Picea abies* and beech *F. sylvatica* interspersed by large clearings for cattle pastures, where more intensive land-use management and grazing pressure keep ecosystem succession in check. Fifteen mesocosms were transplanted at each of the elevations: 1350, 1010, and 570 m a.s.l. with five replicates coming from each of the following three land-use types representing a gradient of land-use intensity, respectively, grazing activities: a densely wooded pasture (DW) with 80% light interception by the canopy and a typical understory of woody plants and forbs, a sparsely wooded pasture (SW) with 40% light interception and a rich community of mountain forbs and feather mosses, and an UW pasture

without light interception and a subalpine grassland community. The respective donor sites were situated within 1 km of each other, each measured about 2 ha, and all shared the same geomorphology, microtopography, and aspect. Prior to the transplantation, the solar radiation field conditions in each land-use type were logged (PYR sensors, Decagon Devices, Inc., USA) over an entire growing season and subsequently simulated at each common garden site by two types of UV-resistant meshed roofs (that is, 40 and 80% shading) suspended over wooden frames at 50 cm above the surface of the mesocosms. The latter did not intercept rain precipitation and were taken off during winter to avoid interference with the snowpack. Due to logistical limitations, only five mesocosms of an UW pasture type were transplanted to 395 m a.s.l. Details of edaphic characteristics for each land-use type are given in Table 1.

Each mesocosm was equipped with a set of ECH_2O EC-TM sensor probes coupled to Em50 data-loggers (Decagon Devices, Inc., USA) recording soil temperature and volumetric water content in top-soil (0 to -3 cm) every minute and averaging data over 1 h intervals. In addition, at each experimental site an automated weather station (Sensor Scope Sàrl, Switzerland) measured rain precipitation and air temperature and humidity 2 m above the ground surface at 1 min intervals.

The decomposition rate along the gradient was evaluated using the litter bag technique (Crossley and Hoglund 1962) and standard leaf litter of European beech *F. sylvatica*. Beech is a common deciduous tree species in European forests and its distribution in Swiss mountains ranges between 400 and 1400 m a.s.l. (Weber and others 2010). We used naturally senesced and shed leaves which were intercepted with meshes suspended at about 1 m above the forest floor within a homogeneous, mature beech stand situated at 580 m a.s.l. in the Arboretum National d'Aubonne N 46°51′, E 6°37′. Leaves were collected in October 2009 without bias toward size, shape or color, and petioles were considered part of the leaf. Following air drying at 20°C until constant weight was reached, approximately 1.5 g litter was enclosed in UV-resistant polypropylene litterbags (10 × 10 cm) of 5-mm-mesh size, and the mass of each bag was recorded. A double layer mesh was used for the bottom of the bags to limit the loss of fragmented litter, but still allowing for the access of macroarthropodes and earthworms (Cornelissen 1996). Subsamples were taken for establishing the initial chemical (carbon and nitrogen) composition and the gravimetric water content of the litter.

Litter bags were incubated on top of the soil after parting the extant vegetation and fixed in place by means of small plastic pegs, one in each corner of the bag. Five individual litter bags were placed in each plot in November 2009 and then collected sequentially in spring 2010, autumn 2010, spring 2011, autumn 2011, and spring 2012. To reflect the difference in winter season length at each altitude, spring sampling took place shortly after snowmelt and the flowering of *Crocus albiflorus* in the mesocosms. Both autumn samplings took place in early October with the onset of vegetation senescence.

Litter Mass-Loss, Chemical, and PLFA Analyses

Collected litterbag samples were brushed of adherent soil and subsequently lyophilized in a freeze drier (CHRIST Beta 2-8 LD plus, Germany) and weighed to determine their dry weight. Litter decomposition was expressed as percentage of initial dry mass-loss over time. A subsample of coarsely fragmented litter was taken for phospholipid fatty acid (PLFA) analysis and the rest of the sample was ground in a ball mill (Retsch MM200, Germany) for estimation of carbon and nitrogen content in an elemental analyzer (Carlo ErbaCNS-O 1108, Italy).

Total microbial biomass, fungal, and bacterial biomass were determined using PLFA biomarkers. The analysis followed the protocol of White and others (1979) with modifications as in Andersen and others (2010). In brief, lipids from 300 ± 1 mg of lyophilized litter were dissolved in a 1:2:0.8 solution of chloroform, methanol, and potassium phosphate buffer and shaken for 2 h. Equal volumes of chloroform and buffer were then added and the two phases were left to separate overnight. The chloroform phase was reduced by N_2 evaporation and the lipids were then split into neutral, glyco-, and phospholipids with silica solid phase extraction cartridges by eluting sequentially with chloroform, acetone, and methanol. The phospholipids were then trans-esterified in fatty acid methyl esters (FAMEs), evaporated under oxygen-free N_2, and re-suspended in 1 ml hexane containing 100 µl of methyl nonadecanoate (19:0) (Sigma-Aldrich, Switzerland) as an internal standard. FAMEs were subsequently identified by retention time in a gas chromatograph (Varian Inc. CP3800, USA) with reference to commercial standards including bacterial and fungal acid methyl esters (Supelco, USA).

We used PLFAs i15:0, a15:0,15:0, i16:0, 16:1ω7c, i17:0, cy17:0, 17:0, and cy19 as markers for

bacteria; and 18:3ω6 and 18:2ω6c as markers for fungi (Bardgett and others 1996; Wilkinson and others 2002). Following de Vries and others (2012), we used the sum of all these biomarkers as an indicator of microbial community size and the ratio between fungal and bacterial biomarkers as an indicator of microbial community structure.

Statistical Analysis

Each response variable of interest (daily soil temperature and moisture, remaining litter mass, litter chemistry, amount, and composition of microbial PLFA) was tested in a mixed effects model for the effect of *land use* as a categorical variable, *altitude* as a continuous covariate, and *sampling date* as a repeated measure, as well as their two- and three-way interactions. We used a maximized restricted log-likelihood (REML) procedure (Pinheiro and others 2012) to account for the unbalanced structure of our dataset, that is, four altitudes in the case of UW land-use type, as opposed to three. As a general rule (Green and Tukey 1960), non-significant interaction terms with *F* values < 2 were pooled with the error term. To elucidate significant interactions with *land use*, the effects of *altitude* and *sampling date* were furthermore tested within each land-use type using the same model structure (Engqvist 2005). In addition, linear regression models were employed to establish the effects of atmospheric climate (mean daily values for air temperature, air humidity, and precipitation) on the respective soil microclimate (soil temperature and moisture) within the transplanted mesocosms.

For each mesocosm, mean values of soil temperature and moisture between two sampling dates were used as representative of the microclimate experienced by litter bags during each incubation period. Their effect on the litter mass loss between successive sampling dates was tested in a similar mixed effect model as described above, using them as continuous covariates. Because all sampling dates tightly followed the winter and summer seasons, we tested the effect of sampling date (that is, first winter, first growing season, second winter, second growing season, and third winter) on the sequential litter mass loss, using sampling date as a categorical variable. Similarly, the sampling dates were grouped into two categories—winter and growing season—to compare the corresponding mass loss in those two contrasting periods.

Comparison of litter decay rates among the different *altitude* and *land-use* treatments was done using the decomposition rate constant (*K* value) from a negative exponential model: $M_t = M_0 e^{-kt}$ (Olson 1963), where M_t is the mass in litter bags at a given sampling date, M_0 is the initial mass in litterbags, and t is the incubation period in years. Individual models were fit to the five sampling dates within each plot and solved for k. The resulting K values were tested in an analysis of covariance for the effects of *land use*, *altitude*, and their two way interaction. Based on our hypothesis for an optimum climatic range for decomposition at mid-elevation, *altitude* was specified in the model both as linear and quadratic terms. As above, upon detection of a significant interaction, the effect of *altitude* was determined within each land-use type separately; and that of *land use*—within each altitude along the gradient.

Assumptions of normality and homoscedasticity of the residuals in all linear regression models were verified visually using diagnostic plots and effects were considered significant at $P < 0.05$. All statistical analyses were performed in R 2.15.1, using the package 'nlme' (Pinheiro and others 2012).

RESULTS

Climatic Gradient

Microclimatic conditions in the transplanted mesocosms varied significantly over the 2.5 year decomposition period (Figure 2). Turf transplantation to lower altitude resulted in a significant increase in soil temperature ($F_{1,44} = 262.2$, $P < 0.001$) and a concomitant decrease in soil moisture ($F_{1,44} = 30.0$, $P < 0.001$). Turfs from the contrasting land-use types were overall warmer ($F_{2,44} = 9.6$, $P < 0.001$) and wetter ($F_{1,44} = 30.0$, $P < 0.001$) with decreasing canopy shading in the order DW < SW < UW pastures (Table 1). The observed gradient in soil surface temperature in the mesocosms was mainly due to variation in the air temperature along the altitudinal transect (linear regression $R^2 = 0.77$, $P < 0.001$), with the small part of unexplained variation coming from the winter periods when snow covered soils remained decoupled from atmospheric temperature fluctuations. Soil moisture, however, was not directly influenced by any of the measured climatic variables (air temperature, air humidity, and precipitation) and showed a significant decrease at lower altitude ($F_{1,18} = 48.5$, $P < 0.001$) only for the UW pasture *land use*, but not for the other two wooded pasture types (Figure 2).

Litter Decomposition Rates and Mass Loss

Overall, the remaining mass in the litterbags decreased significantly at higher altitude ($F_{1,44} = 40.4$, $P < 0.001$) and was significantly less (up to 50%
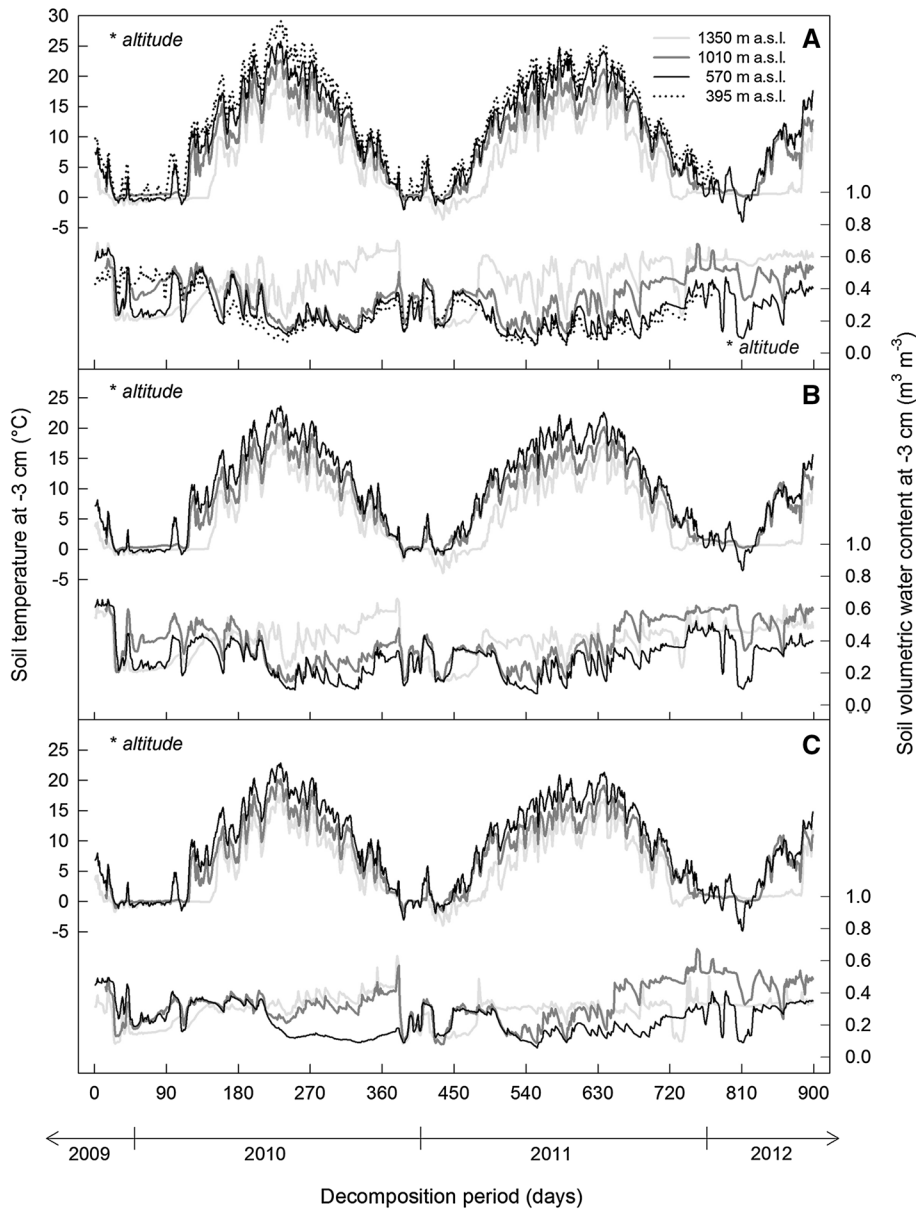
Figure 2. Soil microclimate in the transplanted mesocosms with turfs from: **A** unwooded pasture, **B** sparsely wooded pasture, and **C** densely wooded pasture land-use type, during the course of the 2.5 year long litter decomposition experiment. Presented are mean daily values for five replicate mesocosms. At the lowest altitude only the unwooded pasture land use was considered. *Asterisks* indicate significant effects of the specified factors within each of the land-use types.

mass loss after 2.5 years) in the UW pasture *land use* ($F_{2,44} = 8.5$, $P < 0.001$). Nevertheless, as seen in Figure 3, exclusively the UW pastures experienced distinct rates of mass loss ($F_{1,18} = 44.6$, $P < 0.001$) with respect to *altitude*, which was reflected by a significant *altitude*:*land-use* interaction ($F_{2,44} = 7.9$, $P = 0.001$). Mean soil moisture between two successive sampling dates had a significant effect on the sequential mass loss of litter ($F_{1,161} = 5.4$, $P = 0.021$), but soil temperature had none. Mass loss was comparable between individual sampling dates and no apparent effect of winter or growing season periods was observed either.

All the estimated decay constants (K values) came from significant exponential models with 84% of the R^2 values within the range 0.8–1.0, and no R^2 values below 0.6. Similar to the mass loss analysis, the decomposition rates exhibited strong altitudinal ($F_{1,43} = 14.6$, $P < 0.001$) and land use ($F_{2,43} = 7.8$, $P = 0.001$) patterns, with a significant interaction between these two factors ($F_{2,43} = 8.0$, $P = 0.001$). Figure 4 shows that decomposition was positively affected by *altitude* only within the UW pasture type and this effect was linear ($F_{1,18} = 25.5$, $P < 0.001$). The rates of litter decomposition in the other two wooded land-use types did not vary along the altitudinal gradient, and they exhibited lower decomposition rates than UW at both 1350 m a.s.l. ($F_{2,12} = 6.9$, $P = 0.010$) and 1010 m a.s.l. ($F_{2,12} = 6.9$, $P = 0.011$).
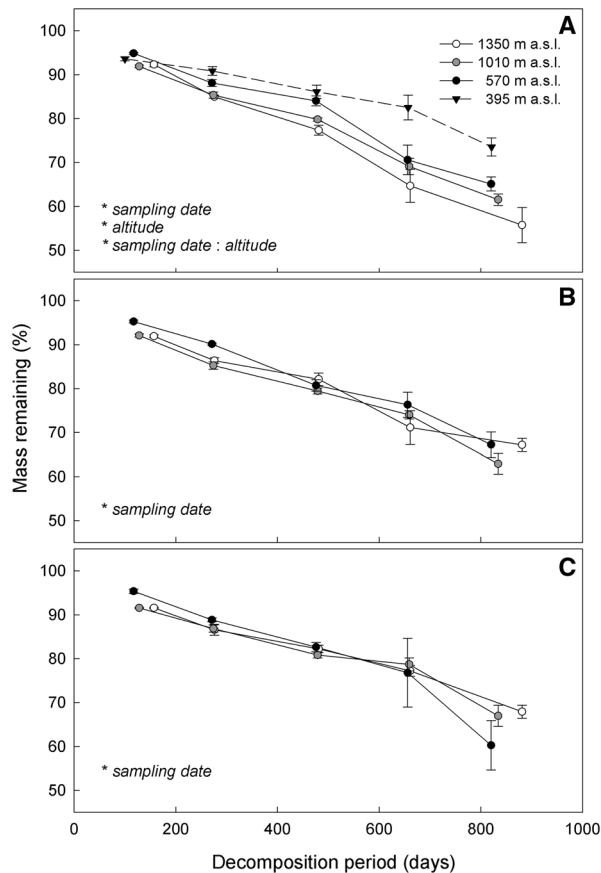
Figure 3. Percentage of initial mass remaining in the litter bags bi-annual sampling intervals, following their incubation in autumn 2009 on the surface of mesocosms from: **A** unwooded pasture, **B** sparsely wooded pasture, and **C** densely wooded pasture land-use type. Presented are mean values and standard errors for litterbags from five replicate mesocosms. At the lowest altitude only the unwooded pasture land use was considered. *Asterisks* indicate significant effects of the specified factors within each of the land-use types.

Litter Chemistry

Overall, the initial stoichiometric ratio of carbon and nitrogen (C/N ratio = 71.3) decreased substantially over the course of litter decomposition (*sampling date*: $F_{1,196} = 605.2$, $P < 0.001$), and was largely influenced by the *altitude* of litter bag incubation ($F_{1,46} = 64.6$, $P < 0.001$) and the *land use* ($F_{2,46} = 3.9$, $P = 0.027$). Figure 5 shows that litter at lower altitudes had a consistently lower C/N ratio across all three land-use types, however, the decrease was most pronounced at the UW pasture land use. When investigated separately, carbon and nitrogen contents revealed that a pulse increase of +40% in nitrogen concentration after the first year of incubation and a steady one
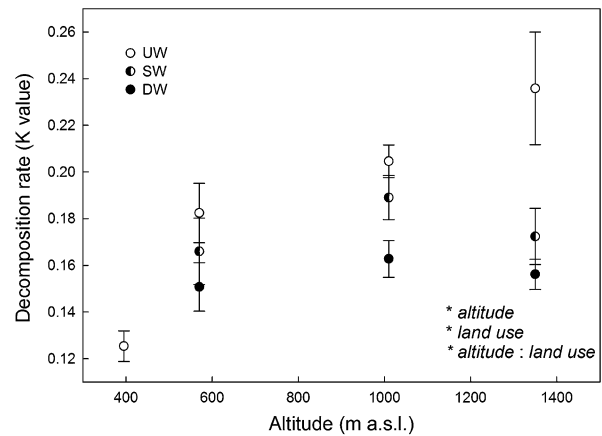

Figure 4. Leaf litter decomposition rates (*K* values) of *Fagus sylvatica* along an altitudinal gradient with mesocosms from three land-use types: unwooded pasture (UW) with 0% canopy shading, sparsely wooded pasture (SW) with 40% canopy shading, and densely wooded pasture (DW) with 80% canopy shading. Presented are mean values and standard errors for five replicate plots. At the lowest altitude only the unwooded pasture land use was considered. *Asterisks* indicate significant effects of the specified factors.

thereafter (*sampling date*: $F_{1,196} = 992.4$, $P < 0.001$) was responsible for this change (see also Supplementary Material, Figure A1). This pattern was most expressed at lower *altitude* ($F_{1,46} = 75.7$, $P < 0.001$).

Microbial Community Composition

The microbial communities on decomposing litter, assessed by the sum of the PLFA markers for fungi and bacteria, grew steadily over the course of the experiment (*sampling date*: $F_{1,198} = 179.7$, $P < 0.001$) without a significant *land use* effect (Figure 6). Only those litter bags incubated in UW pasture mesocosms revealed an *altitude* effect ($F_{1,18} = 7.6$, $P = 0.013$), which, however, varied significantly in its direction over time (*sampling date :altitude* $F_{1,78} = 4.0$, $P = 0.049$) causing either an increase or a decrease in the total amount of PLFAs at a given sampling date.

A seasonal variation in fungal/bacterial ratio was evident too (Figure 7), with a significant overall effect of *sampling date* ($F_{1,197} = 66.7$, $P < 0.001$). The magnitude of the *altitude:sampling-date* interaction ($F_{1, 197} = 8.75$, $P = 0.004$) decreased along the UW–DW pasture gradient and so did the effect of *altitude per se*. Indeed, as in the case of total microbial PLFA, the variation in fungal/bacterial ratio in the litter bags was only influenced by *altitude* within the UW pasture mesocosms ($F_{1,18} = 13.2$, $P = 0.002$). Overall, the *altitude* treatment caused a significant increase in bacterial PLFA markers at lower altitude
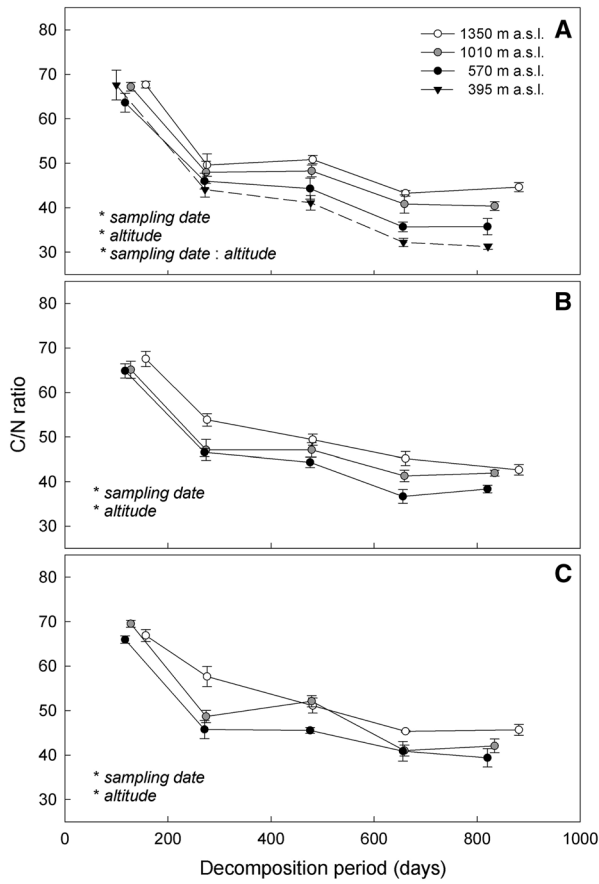
Figure 5. Dynamics of leaf litter C/N ratio during the decomposition period in mesocosms with turfs from: **A** unwooded pasture, **B** sparsely wooded pasture, and **C** densely wooded pasture land-use type. Presented are mean values and standard errors for five replicate mesocosms. At the lowest altitude only the unwooded pasture land use was considered. *Asterisks* indicate significant effects of the specified factors within each of the land-use types.

Figure 6. Microbial biomass on decomposing litter as represented by PLFA markers. Land-use types are: **A** unwooded pasture, **B** sparsely wooded pasture, and **C** densely wooded pasture. Presented are mean values and standard errors for five replicate mesocosms. At the lowest altitude only the unwooded pasture type was considered. *Asterisks* indicate significant effects of the specified factors within each of the land-use types.

($F_{1,46} = 17.3$, $P < 0.001$), but did not affect the fungal ones.

DISCUSSION

Climate and Land-Use Driven Patterns in Litter Decomposition

Decomposition rates over the two and a half year incubation period were within the range of most commonly reported K values for leaf litter on a global basis, that is, 0.1 to 0.3 (Zhang and others 2008). Based on the type of land use, the mass loss from *F. sylvatica* litterbags showed distinct trajectories in its response to the climate along the altitude gradient. Only in the non-shaded UW mes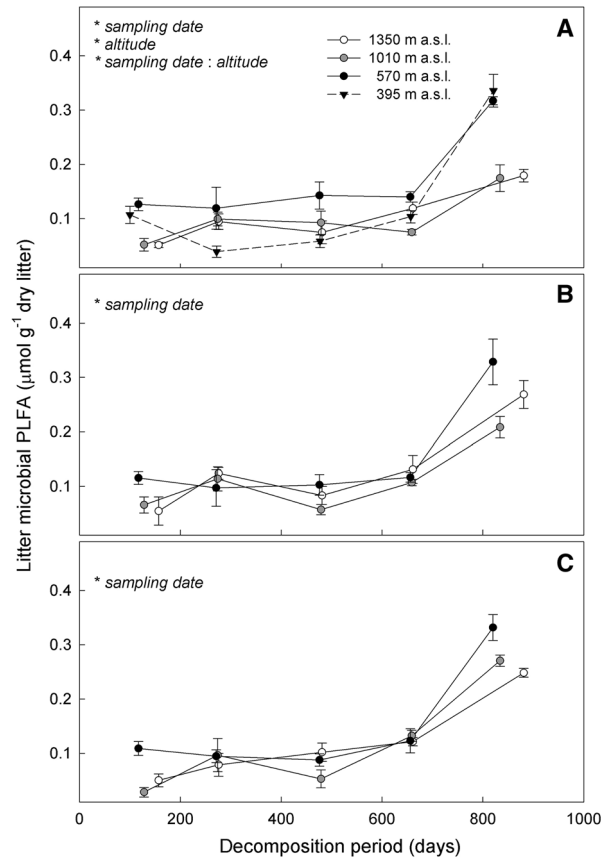ocosms were the decay rates affected by the altitude treatment, with higher rates at higher altitude. Such a pattern was previously reported for altitudinal transects across vegetation zones in North American (Murphy and others 1998) and European (Duboc and others 2012) mountains, but never, to our knowledge, for transplanted turfs, that is, under standardized soil conditions. Contrary to our hypothesis about a local optimum range for litter decomposition at mid-elevation, based on the offset between air temperature and precipitation along the climatic gradient, we observed either a clear linear relationship with altitude (UW land use), or no such at all (SW and DW land use). Indeed, our data provided evidence for a prevailing moisture limitation on the decomposition process. Temperature did not act as a constraint on litter decay at higher altitude, however, soil moisture exerted a strong control at lower
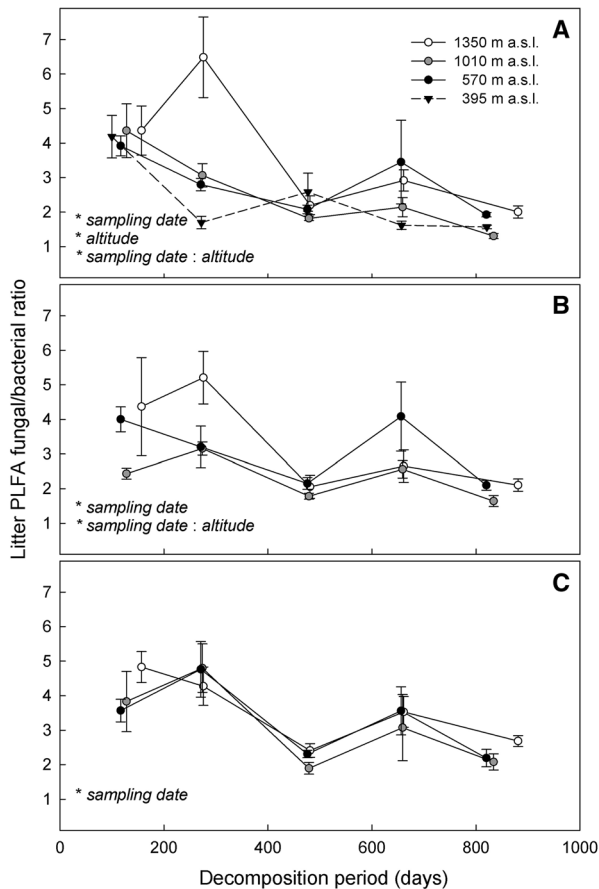
Figure 7. Ratio of fungal to bacterial PLFA markers in the litter bags. Land-use types are: **A** unwooded pasture, **B** sparsely wooded pasture, and **C** densely wooded pasture. Presented are mean values and standard errors for five replicate mesocosms. At the lowest altitude only the unwooded pasture type is considered. *Asterisks* indicate significant effects of the specified factors within each of the land-use types.

altitude. Indeed, both litter mass loss and soil moisture decreased significantly with warmer and drier climate at lower altitude within the UW mesocosms. In contrast, within the two shaded land-use types (SW and DW), where no change in decomposition rates was detected in relation to altitude, temperature was consistently warmer at lower altitudes, but soil moisture experienced an idiosyncratic seasonal pattern, with no consistent altitudinal gradient over the entire incubation period. These results suggest a strong point about the role of soil moisture as a driving factor of leaf litter decomposition and furthermore neatly demonstrate the importance of vegetation type (that is, land use, canopy cover, and so on) for modulating evapotranspiration and subsequently decomposition rates (see Aerts 2006). A relevant process

taking place on the surface of the mesocosms is the net difference in radiative heating among the three land-use types. Due to their shading, each of the two "wooded" treatments experienced, on average, cooler temperatures than the UW one. Hence, taking into account the intensified evapotranspiration at warmer air and/or soil temperatures (Seneviratne and others 2006), UW mesocosms were much more prone to soil moisture limitation during persistent warm and dry periods. At the landscape scale, such a dichotomy in the response of grasslands and forests to heat waves was demonstrated too (Teuling and others 2010), providing evidence for a more conservative use of water by forests, which preserves soil moisture in the long term and prevents excessive heating due to drought.

Our five biannual samplings allowed us to study the intra-seasonal decomposition pattern and the observed steady decay rates provided evidence for comparable mass loss across seasons. On the one hand, this agrees with previous studies demonstrating substantial mass loss rates during the winter season (Sjögersten and Wookey 2004; Baptist and others 2010; Saccone and others 2013). On the other hand, it is in contrast to the stipulated model of faster and slower stages of litter decomposition based on a decreasing litter quality over the course of the decomposition process (Couteaux and others 1995). This could be tentatively explained by the principle that the early stages of decay are substrate quality driven and the later stages are microbial community composition driven (Bray and others 2012), such that the interplay between those and the seasonal change in climate and microbial structure (see Bardgett and others 2005; and the discussion below) has resulted in the observed steady decomposition rate over the entire incubation period.

Microbial Community Shifts in Relation to Litter Chemistry and Decomposition Along the Altitudinal Gradient

Plant litter chemistry changed substantially over the course of time, with litter C/N ratio decreasing both as decomposition progressed and in response to the altitudinal treatment. The observed change in C/N ratio was exclusively due to an increase in N concentration in the litter, following microbial immobilization of nitrogen from the soil (Melillo and others 1982; Parton and others 2007). Because litter quality and N content, in particular, have a dominant role in determining decomposition rates globally (Cornwell and others 2008), recalcitrant

litter types (such as *F. sylvatica*) require that allochthonous N is brought up by microbes to access the C in the litter (Rutigliano and others 1998). In this study, the process occurred after 1 year of incubation, during the first plant growing season and not during the preceding winter (see Figures 5, A1), indicating a prior limitation in N-availability and/or limited colonization by microbial decomposers (see also Figure 6) during the first winter. Furthermore, the disproportional decrease in litter C/N within mesocosms at lower altitudes could be due to a general increase in N-availability resulting from warmer soil temperatures. Indeed, we found a concomitant increase of N-mineralization rates and leaching of N into the soil solution at lower altitudes (Supplementary Material, Table A1)—a trend which has been previously established for both altitudinal gradients (Zhang and others 2012) and globally in warming experiments (Bai and others 2013). The land-use pattern of lower C/N ratios in litterbags incubated in UW mesocosms could be further attributed to the synergistic effect of extant labile grassland litter (that is, lower soil organic matter C/N ratio, Table 1) on the decomposition rates of *F. sylvatica*, allowing the transfer of N between the two (see Schimel and Hättenschwiler 2007).

Alongside the decrease in litter C/N stoichiometry, a change in microbial community structure took place, resulting in a matching decrease in fungal/bacterial ratios in the litter bags during the period of decomposition. In line with the decrease in fungal/bacterial ratios reported with increased management intensity, increased nutrient availability, and early successional stage (van der Heijden and others 2008), we interpret our results for a decrease in fungal/bacterial ratio at lower altitude (especially in the UW mesocosms) as an alteration of the nutrient cycle. This follows from the general concept for an increasing importance of bacteria in ecosystems experiencing a faster nutrient cycle (Wardle and others 2004). Hence it is very likely that the observed increase in both N-mineralization rates and concentration of organic and inorganic forms of N in the soil solution at lower altitude caused the shift toward a microbial community rich in bacteria.

Furthermore, the observed temporal dynamics in fungal/bacterial ratio could be attributed to the change in microbial community structure with respect to seasonality, as reported by Schmidt and Lipson (2004). The oscillating pattern, clearly seen in the two wooded pasture land-use types (Figure 7) can be explained by the principle that senesced litter in autumn is colonized by fungi responsible for its degradation over winter, and the subsequent increase in N-availability in the soil solution in spring results in a substantial immobilization by bacteria. Our results suggest that this process in the soil microbial community can be severely disrupted by altered climate as seen in the UW land use, but also that in wooded pastures characterized by a more buffered microclimate and edaphic conditions the decomposer communities experience a much lower impact from climatic perturbation.

CONCLUSIONS

The spatial and temporal continuum of leaf litter decomposition rates presented with this experiment demonstrated the potential of climate gradient studies for the detection of causal relationships between ecosystem processes and climate variability. The linearity in the response of litter decomposition within UW pastures to climate change revealed the hierarchical controlling role of soil moisture on this process. Surprisingly, we did not find evidence for temperature limitation on this process. The observed land-use driven response of litter decomposition to climate has potentially important implications for carbon cycling under climate change. Besides exerting a direct microclimatic effect on litter decomposition, wooded pastures also supported a community of microbial decomposers resistant to climate change perturbation and sustained its functional composition and activity.

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