Oecologia (2010) 163:247–256
DOI 10.1007/s00442-009-1528-1

GLOBAL CHANGE ECOLOGY - ORIGINAL PAPER

The importance of biotic factors in predicting global change effects on decomposition of temperate forest leaf litter

Soraya Rouifed · I. Tanya Handa · Jean-François David · Stephan Hättenschwiler

Received: 10 April 2009 / Accepted: 23 November 2009 / Published online: 24 December 2009

Abstract Increasing atmospheric CO_2 and temperature are predicted to alter litter decomposition via changes in litter chemistry and environmental conditions. The extent to which these predictions are influenced by biotic factors such as litter species composition or decomposer activity, and in particular how these different factors interact, is not well understood. In a 5-week laboratory experiment we compared the decomposition of leaf litter from four temperate tree species (*Fagus sylvatica, Quercus petraea, Carpinus betulus* and *Tilia platyphyllos*) in response to four interacting factors: elevated CO_2-induced changes in litter quality, a 3°C warmer environment during decomposition, changes in litter species composition, and presence/absence of a litter-feeding millipede (*Glomeris marginata*). Elevated CO_2 and temperature had much weaker effects on decomposition than litter species composition and the presence of *Glomeris*. Mass loss of elevated CO_2-grown leaf litter was reduced in *Fagus* and increased in *Fagus*/*Tilia* mixtures, but was not affected in any other leaf litter treatment. Warming increased litter mass loss in *Carpinus* and *Tilia*, but not in the other two litter species and in none of the mixtures. The CO_2- and temperature-related differences in decomposition disappeared completely when *Glomeris* was present. Overall, fauna activity stimulated litter mass loss, but to different degrees depending on litter species composition, with a particularly strong effect on *Fagus*/*Tilia* mixtures (+58%). Higher fauna-driven mass loss was not followed by higher C mineralization over the relatively short experimental period. Apart from a strong interaction between litter species composition and fauna, the tested factors had little or no interactive effects on decomposition. We conclude that if global change were to result in substantial shifts in plant community composition and macrofauna abundance in forest ecosystems, these interacting biotic factors could have greater impacts on decomposition and biogeochemical cycles than rising atmospheric CO_2 concentration and temperature.

Keywords Elevated carbon dioxide · Litter diversity · Litter quality · Saprophagous macrofauna · Temperature

Communicated by Amy Austin.

S. Rouifed · I. T. Handa (✉) · J.-F. David · S. Hättenschwiler
Centre d'Ecologie Fonctionnelle et Evolutive (CEFE),
CNRS, 1919 route de Mende, 34293 Montpellier, France
e-mail: tanya.handa@cefe.cnrs.fr

Present Address:
S. Rouifed
Laboratoire d'Ecologie des Hydrosystèmes Fluviaux,
UMR CNRS 5023, Université de Lyon 1,
69622 Villeurbanne, France

Introduction

Multiple facets of global change including rising atmospheric CO_2 concentrations, climate warming and the loss of biodiversity are predicted for this century (Sala et al. 2000; IPCC 2007). These changes are expected to affect ecosystem processes, for example through alterations to the terrestrial C cycle, changes in community composition and potential resulting feedbacks (Körner 2000; Loreau et al. 2001; Pendall et al. 2004; Hector and Bagchi 2007). Forests, which store about 85% of the global biomass C (Saugier et al. 2001), are particularly important and much debate has surrounded the ecosystem function of litter decomposition, which mediates the transfer of dead organic matter into the soil C pool (Lavelle and Spain 2001; Chapin et al. 2002; Sayer 2006). The rate of litter decomposition is

controlled predominantly by climate, litter quality and soil organisms (Heal et al. 1997), which all may be liable to change directly or indirectly in the coming decades in response to the above-mentioned factors (Coûteaux et al. 1995; Wardle 2002; Bardgett 2005; Aerts 2006).

Elevated atmospheric CO_2 concentrations typically modify litter quality, as summarized in a meta-analysis by Norby et al. (2001), who showed an overall 7.1% reduction in N concentration coupled with a 6.5% increase in lignin concentration. This altered litter quality is hypothesized to slow down decomposition, although Norby et al. (2001) report that across all studies in their meta-analysis, no overall significant effect on litter mass loss was observed. Conversely, in response to increasing temperatures, decomposition processes are expected to accelerate as a result of an increased microbial activity under sufficiently moist conditions (Coûteaux et al. 1995; Robinson 2002; Aerts 2006), although temperature sensitivity of decomposition can vary depending on the litter type (Fierer et al. 2005). The interaction between rising atmospheric CO_2 concentration and temperature is critical for responses at the ecosystem level, because of expected contrasting effects on decomposition and subsequent consequences for the C and N cycles (Pendall et al. 2004; Hyvönen et al. 2007; Wan et al. 2007).

The functional role of biodiversity is equally important for reliable estimates of decomposition rates in species-rich forest ecosystems (Wardle 2002; Bardgett 2005; Hättenschwiler et al. 2005). For example, mass loss in mixtures of litter from co-occurring species is often not predictable from mass loss of component litter species decomposing individually (Gartner and Cardon 2004; Hättenschwiler et al. 2005). Apparent synergistic effects in litter mixtures typically lead to faster than expected decomposition rates, while antagonistic effects resulting in slower than expected decomposition rates occur less frequently (Gartner and Cardon 2004). Positive effects of litter mixtures on decomposition may be partly driven by litter-feeding invertebrates benefiting from a more nutritionally diverse diet (Hättenschwiler and Gasser 2005).

Soil fauna play a fundamental role in the regulation of decomposition processes, as recently demonstrated in a global decomposition experiment (Wall et al. 2008). Where saprophagous macrofauna, such as earthworms, woodlice and millipedes, are abundant, they have major effects on decomposition via fragmentation, gut processing and translocation of the litter material (Schaefer 1991; Wolters 2000; Lavelle and Spain 2001). Changes in litter quality associated with CO_2 enrichment of trees can impact their feeding behaviour, leading either to increased or decreased consumption rates depending on the litter species (Cotrufo et al. 1998; Hättenschwiler et al. 1999; David et al. 2001). The presence of macrofauna may even reverse initially negative CO_2 effects on decomposition (Coûteaux et al. 1991). Large soil invertebrates are responsive to climate warming and their abundance could increase in many ecosystems of cold and temperate areas (Aerts 2006; David and Gillon 2009). However, related to the common use of litterbags of mesh sizes typically between 1 and 2 mm, macrofauna are often excluded from decomposition studies and from biogeochemical models assessing global change impacts on the future C cycle (see Wall et al. 2008).

Influences of atmospheric CO_2 concentration, temperature, litter diversity and soil fauna on decomposition seem to be inextricably linked. However, despite all the data available on interactions between some of these factors, temperature is rarely considered in combination with litter diversity and presence of soil fauna. Furthermore, a decomposition experiment involving all four factors and assessing interactions among them has never been done. In this first-ever study combining all factors, we examined the effects of CO_2-induced litter quality changes, temperature, litter composition and presence of saprophagous macrofauna on mass loss and C mineralization of leaf litter of contrasting quality. Under controlled laboratory conditions, we estimated the relative importance of the four factors and their potential interactions over a 5-week period.

Materials and methods

Plant and animal material

Leaf litter was collected in a 120-year-old mixed deciduous forest at the Swiss Canopy Crane free air CO_2 enrichment research site near Basel, Switzerland (47°28′N, 7°30′E; see Körner 2000; Pepin and Körner 2002; Körner et al. 2005 for details). The forest area accessible by crane (2,830 m^2) has a canopy height of 30–36 m and is dominated by *Fagus sylvatica* L. and *Quercus petraea* L. Less frequent species include *Carpinus betulus* L., *Quercus robur* L., *Tilia platyphyllos* Scop., *Acer campestre* L., and *Prunus avium* L. Since autumn 2000, fourteen trees in the crane-accessible area have been exposed to an elevated CO_2 concentration (ca. 530 µmol mol^{-1}) using a web-free air CO_2 enrichment system (Pepin and Körner 2002). The remaining 50 trees within the crane radius serve as non-enriched controls (ambient CO_2 concentration of ca. 370 µmol mol^{-1}). Leaf litter from both CO_2 conditions was collected directly from the canopy in autumn 2002, 2004 and 2005 by gently shaking branches of multiple trees. Litter was subsequently dried at 40°C and stored dry to prevent any microbial activity. Four species were chosen along a decomposability gradient that was previously determined by Hättenschwiler and Gasser (2005), i.e. in increasing

order of mass loss rate, *F. sylvatica*, *Q. petraea*, *C. betulus* and *T. platyphyllos*. All litter harvests were pooled for each species.

Before the experiment, the stock of dead leaves was placed for a short period on the ground of a *Quercus ilex* forest in Montpellier, southern France. This initial field exposure was carried out for three reasons: it permitted microbial colonization of the litter that had been in storage for some years; it permitted us to work with a substrate that was more representative of the majority of forest litter throughout the year, which has been subject to at least one rainfall event; and soil macrofauna effects on decomposition only start some time after leaf litter fall, on leaves already leached and colonized by micro-organisms (David and Gillon 2002). Litter bags of 36 cm^2 g^{-1} of litter and with a 8-mm mesh allowing free entry of soil animals, were deposited on the ground for either 14 (for the rapidly decomposing litter of two species, *Carpinus* and *Tilia*) or 42 days (for the slowly decomposing litter of two species, *Fagus* and *Quercus*). During field exposure, litter remained structurally intact but all species lost ca. 10% of their initial mass, presumably mainly through leaching. Before the experiment, litter was dried at 40°C and every leaf was individually cleaned of soil particles.

The millipede *Glomeris marginata* (Villers) was chosen as a representative of the saprophagous macrofauna because it was the most abundant species at the Swiss study site. This species is also common throughout northwest Europe (David 1999). Specimens were collected in December 2006 from Mediterranean forests near Montpellier and were kept in plastic boxes with moist soil and leaf litter from their forest sites.

Initial litter quality

After field exposure, leaf litter material was analysed for N, C, lignin and P. Total N and C were measured using a CHN analyser (EA1112; Thermo-Finnigan, Milan). Lignin concentration was measured by the FIBERSAC method adapted from Van Soest's protocol, where lignin is isolated in three successive hydrolysis steps (Loranger et al. 2002). P concentration was determined spectrophotometrically using an automatic colorimetric chain (Evolution II; Alliance Instruments, Frépillon, France). For the calculation of specific leaf area (SLA), leaves were measured with an area metre (MK2; Delta-T, Cambridge, England).

Experimental set-up

Two hundred and sixty microcosms (transparent plastic boxes of about 400 cm^3) were filled with 115 g of sand (a 0.5-cm-thick layer) and 3 g dry mass (±0.01 g) of remoistened, leaf litter material. Pure siliceous sand was chosen as a substrate because of its negligible content of organic matter. The sand was sieved to 2 mm, abundantly rinsed and dried at 50°C for 48 h. Leaf litter consisted either of only one species (monocultures) or of all possible combinations of two or three species from our four-species pool, except those including both *Fagus* and *Quercus*, which show no net mixture effects under field conditions (unpublished data). For mixtures, equal amounts of each litter species were combined in the microcosms. Overall there were 11 different litter types (4 monocultures + 5 mixtures of two species + 2 mixtures of three species) of either ambient CO_2- or elevated CO_2-grown litter. Each litter treatment was applied to 12 microcosms, except for the *Carpinus*/*Tilia* mixture where insufficient ambient CO_2 litter material resulted in only eight microcosms being possible. Half of the microcosms were then placed in three light- and temperature-controlled cabinets (LMS, Sevenoaks, England) at an average temperature of 15°C (17°C for the 12-h light period and 13°C for the 12-h dark period), and the other half in three similar cabinets at an average temperature of 18°C (20°C for the 12-h light period and 16°C for the 12-h dark period). After 48 h of equilibration, two millipedes were added to half of the six microcosms per litter treatment and per temperature treatment, the other three remaining without macrofauna. Individuals were chosen to approach a *Glomeris* biomass of 300 mg per microcosm (±100 mg maximum), so that litter quantity was not limiting for the duration of the experiment [see David and Gillon (2002) for weight-specific consumption rates]. Half-way through the experiment, dead millipedes (<10% of all microcosms) were replaced by animals of the same weight. Every 3 days, microcosms were remoistened with distilled water to ensure consistently high humidity conditions across treatments.

Data collection and analyses

The experimental incubation period was 36 days following the introduction of macrofauna into microcosms. After retrieving millipedes, litter was rinsed with distilled water above the microcosms to wash faeces and sand particles back into the substrate. Litter fragments, nearly all of which were easy to identify, were separated into species, dried at 60°C for 2 days and weighed. The substrate (sand plus faeces and leached organic matter) was dried at 60°C, ground in a ring mill and analysed for total C and N using a CHN analyser (see above).

Litter mass loss was calculated for each microcosm as the percentage of initial dry mass lost during the experiment. C mass loss from each microcosm was calculated as the difference between the amount of C in the litter mass lost during the experiment and the amount of C recovered from the substrate at the end of the experiment (in faeces,

leachates and microbes). This estimate of C loss was expressed as a percentage of the initial amount of C in the litter and used as a proxy for C mineralization over the duration of the experiment. We chose to distinguish between overall litter mass loss and C mineralization mainly to mechanistically interpret the fauna effect on decomposition, which could be a direct effect through litter consumption or an indirect effect through the stimulation of microbial activity in faeces, or a combination of both.

Differences in litter quality parameters among tree species and CO_2 treatments were tested using two-way ANOVAs. Multi-way ANOVAs were used to test for the effects of CO_2 (two levels), temperature (two levels), litter type (11 levels), and presence of macrofauna (two levels) on litter mass loss and C mineralization (main effects and all interactions). Additional analyses were performed to focus on specific points, such as the effect of *Glomeris* on litter mass loss within a litter type (*t*-tests) and the effects of CO_2 and temperature on litter mass loss within a litter type without macrofauna (two-way ANOVAs). When sample variances were unequal, data were transformed to meet assumptions. Post hoc pairwise comparisons were performed using Tukey honest significant difference. The software R, version 2.5.0 was used for all statistical analyses (R Development Core Team 2005).

Results

Initial litter quality

Initial quality of leaf litter differed strongly among tree species for all measured parameters (Table 1, $P < 0.001$), except for SLA (SLA, $P = 0.2$). Litter N concentration ranged from 0.67% in *Fagus* to 2.35% in *Tilia* with increasing concentrations in the order of: $Fagus^a < Quercus^a < Carpinus^b < Tilia^c$ (different superscripts indicate significant differences). *Fagus* and *Quercus*, the two N-poor species, also showed higher C concentrations than *Carpinus* and *Tilia*, resulting in roughly twice as high C:N ratios in the two former species compared to the latter. C:N ratio decreased in the same order of: $Fagus^a > Quercus^a > Carpinus^b > Tilia^c$. The lowest *P* concentration was measured in *Quercus*, with increasing concentrations in the order of: $Quercus^a < Fagus^b < Carpinus^c < Tilia^c$. Although *Tilia* showed the highest nutrient concentrations compared to the other studied species, its lignin concentration was rather high. In fact, lignin concentrations decreased in the order of: $Fagus^a > Tilia^b > Quercus^b > Carpinus^c$ (Table 1).

Atmospheric CO_2 concentrations affected leaf litter quality in some but not all the species (Table 1). *Fagus* leaf litter grown under elevated CO_2 had a reduced N concentration ($P < 0.05$) and a higher C:N ratio ($P < 0.001$); in addition, there was a strong CO_2 effect on SLA, which was 26% lower in elevated-CO_2 compared to ambient-CO_2 litter ($P < 0.05$). *Tilia* leaf litter grown under elevated CO_2 had higher SLA ($P < 0.001$), higher concentrations of N ($P < 0.001$) and C ($P < 0.001$) but lower C:N ratio ($P < 0.001$) than litter from ambient CO_2. There were no significant CO_2 effects on *Carpinus* and *Quercus* litter quality, except for a higher *P* concentration in elevated CO_2-grown *Quercus* litter ($P < 0.01$).

Treatment effects on litter mass loss

The complete model of analysis of variance including all factors and their interactions revealed highly significant main effects of litter type and macrofauna on litter mass loss (Table 2; Fig. 1a). Also, the interaction between litter

Table 1 Quality of leaf litter used for the experiment. N, C, lignin and P concentrations, and specific leaf area (*SLA*) are shown for the four studied tree species grown at ambient or elevated CO_2 (mean ± SE)

	N (%)	C (%)	C/N	Lignin (%)	P (%)	SLA ($cm^2\ g^{-1}$)
Fagus						
Ambient	0.74 ± 0.01	49.2 ± 0.1	67 ± 0	31.1 ± 1.0	0.035 ± 0.001	174 ± 12
Elevated	0.67 ± 0.01	49.2 ± 0.0	74 ± 1	33.5 ± 2.8	0.047 ± 0.007	128 ± 5
Quercus						
Ambient	0.72 ± 0.01	49.2 ± 0.2	68 ± 1	19.0 ± 0.3	0.027 ± 0.001	138 ± 10
Elevated	0.74 ± 0.01	49.7 ± 0.1	67 ± 1	18.8 ± 0.4	0.032 ± 0.001	134 ± 7
Carpinus						
Ambient	1.69 ± 0.07	48.3 ± 0.2	29 ± 1	13.5 ± 1.0	0.089 ± 0.003	150 ± 11
Elevated	1.58 ± 0.01	48.2 ± 0.1	30 ± 0	11.4 ± 0.3	0.084 ± 0.002	141 ± 9
Tilia						
Ambient	1.61 ± 0.02	46.8 ± 0.4	29 ± 0	21.0 ± 0.1	0.082 ± 0.001	132 ± 2
Elevated	2.35 ± 0.02	49.7 ± 0.2	21 ± 0	23.6 ± 3.2	0.120 ± 0.010	156 ± 15

Table 2 ANOVA to test for the effects of CO_2, temperature, litter type and macrofauna on leaf litter mass loss and C mineralization

Source of variation	Litter mass loss			C mineralization		
	F	*P*	*df*	*F*	*P*	*df*
CO_2	0.92	0.34	1	0.26	0.61	1
Temperature (*T*)	0.73	0.39	1	0.49	0.48	1
Litter type	68.43	**<0.001**	10	50.84	**<0.001**	10
Macrofauna	76.50	**<0.001**	1	0.21	0.65	1
$CO_2 \times T$	0.29	0.59	1	0.06	0.81	1
$CO_2 \times$ Litter	1.08	0.38	10	1.74	0.07	10
$T \times$ Litter	0.68	0.74	10	1.03	0.42	10
$CO_2 \times$ Fauna	2.14	0.14	1	5.88	**0.02**	1
$T \times$ Fauna	0.21	0.65	1	0.09	0.77	1
Litter × Fauna	5.26	**<0.001**	10	2.32	**0.01**	10
$CO_2 \times T \times$ Litter	0.86	0.57	10	0.65	0.77	10
$CO_2 \times T \times$ Fauna	1.43	0.23	1	0.26	0.61	1
$CO_2 \times$ Litter × Fauna	1.17	0.31	10	1.65	0.10	10
$T \times$ Litter × Fauna	2.01	**0.03**	10	1.97	**0.04**	10
$CO_2 \times T \times$ Litter × Fauna	0.93	0.51	10	1.67	0.09	10

Significant effects are indicated in *bold*

type and macrofauna accounted for a significant amount of variability, and this interaction was further influenced by temperature (Table 2).

The main effect of litter type reflected the large differences in mass loss among the four studied species in monocultures. Mass loss increased in the order of *Fagus*^{a} < *Quercus*^{a} < *Carpinus*^{b} < *Tilia*^{c} (Fig. 1a), which matched well the decreasing C:N ratio ($r^2 = 0.88$, $P < 0.05$). Also, combinations of species showed higher mass loss in litter mixtures with increasing proportions of rapidly decomposing species (Fig. 1a). The main effect of macrofauna reflected the generally higher litter mass loss when *Glomeris* was present in microcosms. This stimulating effect, however, was highly dependent on the litter type, as indicated by the significant interaction term (Table 2). Mass loss of *Tilia* monocultures increased by 20% in the presence of *Glomeris* ($P < 0.01$), while mass loss of *Carpinus*, *Fagus* and *Quercus* monocultures was not significantly affected by the millipede (Fig. 1a). Also, when *Tilia* was absent from litter mixtures, *Glomeris* showed no significant effect on litter mass loss. Even though there was a general positive effect of *Glomeris* on mass loss of litter mixtures including *Tilia*, this effect varied considerably depending on the other litter species within the mixture. *Glomeris* increased litter mass loss by 58% when *Fagus* was combined with *Tilia* (and by 55% in the triple combination with *Carpinus*), but only by 18% when *Carpinus* alone was combined with *Tilia* (Fig. 1a).

Compared to the strong effects of litter type and macrofauna, both the effect of elevated CO_2 via changes in litter quality, and the direct effect of elevated temperature during decomposition, were subtle. The observed second-order interaction between temperature, litter type and macrofauna reflected that any temperature effect not only depended on litter type, but disappeared completely when *Glomeris* was present. A closer look at the data from microcosms without macrofauna revealed some temperature and CO_2 effects in certain litter types (Fig. 2). A higher temperature during decomposition increased mass loss in the two relatively fast-decomposing species, *Carpinus* (+28%, $P = 0.05$) and *Tilia* (+30%, $P < 0.05$), whereas mass loss of *Fagus* and *Quercus* did not change significantly. However, in *Fagus*, the temperature effect actually depended on CO_2, which overall slowed litter mass loss by 49% ($P < 0.01$, Fig. 2). With a 3°C warmer air temperature during decomposition, this negative CO_2 effect in *Fagus* tended to be greater ($P = 0.08$ for the $CO_2 \times$ temperature interaction). Mass loss in the *Fagus*/*Tilia* mixture was also influenced by elevated CO_2, but positively (+23%; $P < 0.05$).

Treatment effects on C mineralization

C mineralization (C loss from the system over the 5-week period) represented 5–23% of the initial litter C mass placed in the microcosms (Fig. 1b). The complete model analysis revealed that litter type was the single significant main factor influencing C mineralization (Table 2). The presence of *Glomeris* did not significantly affect C mineralization, in contrast to mass loss. There was, however, a significant interaction between litter type and macrofauna (Table 2). The *Carpinus*/*Tilia* mixture showed 32% less C mineralization in the presence of *Glomeris*, whereas the millipede had no effects in the other litter types (Fig. 1b). This observation also likely explains the significant second-order interaction between litter type, macrofauna and temperature (Table 2). Finally, there was an overall positive effect of elevated CO_2 on C mineralization (+9%) in microcosms without macrofauna, and a slightly negative effect (−5%) in the presence of *Glomeris*, which explains the significant interaction between CO_2 and macrofauna (Table 2).

Discussion

Our study is the first to have experimentally tested the interactive effects of CO_2-driven litter quality changes, increased temperature, differing litter types, and the presence of litter-feeding macrofauna on litter decomposition as a key ecosystem process. The results demonstrate strong main effects of the identity of litter species, in isolation or in litter mixtures, and of the presence of millipedes, but

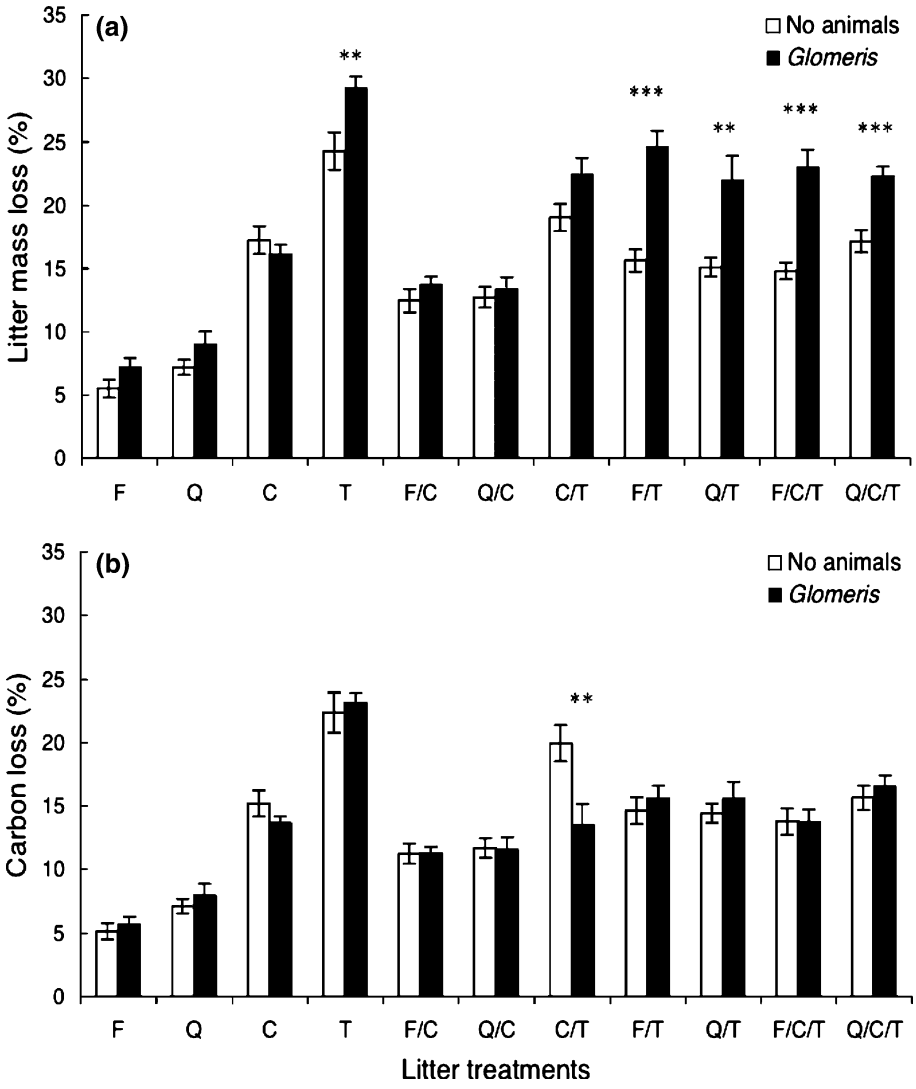

Fig. 1 Loss of **a** litter mass in % of initial mass and **b** C in % of initial C (proxy for C mineralization) for each litter type [*Fagus* (*F*), *Quercus* (*Q*), *Carpinus* (*C*) and *Tilia* (*T*), plus seven mixtures] used in the test. Data (means ± SE) in the absence (*white bars*) or presence (*black bars*) of macrofauna are shown. All data are pooled across CO_2 and temperature treatments. $^{**}P < 0.01$, $^{***}P < 0.001$ (significant effect due to *Glomeris* presence)

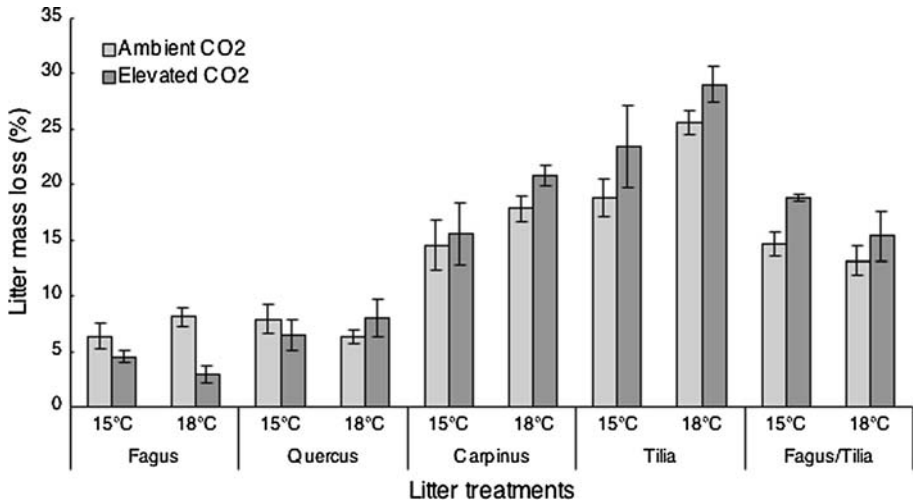

Fig. 2 Litter mass loss in the absence of macrofauna in the two temperature treatments for microcosms containing ambient CO_2-grown and elevated CO_2-grown litter (% of initial mass, mean ± SE). Data of all four monocultures and the mixture *Fagus/Tilia* (the only mixture with a significant effect of at least one of the two factors) are shown

comparatively small effects of CO_2-induced changes in litter quality and higher temperatures. Moreover, there is little evidence for interactions among the tested factors, except for a strong interaction between litter species composition and macrofauna effects. These results raise the question of whether biotic factors are sufficiently taken into account when predicting the effects of global change on decomposition and biogeochemical cycles.

Influences of elevated CO_2 and temperature

Analyses of initial litter quality showed that CO_2 enrichment increased the C:N ratio in *Fagus* while decreasing it in *Tilia*, a trend also reported by Hagedorn and Machwitz (2007) for leaf litter from the same experimental site. CO_2 enrichment does not change litter trait parameters uniformly across species and changes, if observed, do not always result in an effect on decomposition (Norby et al. 2001; Bradley and Pregitzer 2007). In the present study, some effects on mass loss were observed, but only in the absence of *Glomeris*. *Fagus* litter produced under elevated CO_2, with the higher C:N ratio, decomposed more slowly in monocultures than litter produced under ambient CO_2. This result from a short-term experiment under laboratory conditions confirms previous results from a 331-day field experiment with litter from the same species (Hättenschwiler et al. 1999). Slower decomposition due to CO_2-induced changes in litter quality has been documented for other temperate deciduous forest species such as *Betula pendula*, *Betula papyrifera* and *Populus tremuloides* (Cotrufo and Ineson 1996; Parsons et al. 2008). The lack of CO_2 effects in our three other species has also been found for various tree species in previous studies (Finzi et al. 2001; Cotrufo et al. 2005). In the absence of *Glomeris*, we even observed a trend towards greater mass losses in elevated CO_2 *Tilia* monocultures and a significant 23% increase in mass loss when *Tilia* was added to *Fagus*. This reversal driven by *Tilia* is a reminder of how effects in monocultures can be completely changed by the addition of other co-occurring species (Gartner and Cardon 2004; Hättenschwiler et al. 2005). Mechanistically, species richness can increase microbial abundance and enzymatic activity, which can be further amplified by elevated CO_2 (Chung et al. 2006, 2007). The species- and litter mixture-specific responses observed here and elsewhere suggest that potential consequences of CO_2-mediated litter quality changes for ecosystem C and nutrient cycles depend strongly on relative tree species abundances and community composition.

Although temperature is known as a key driver of decomposition (Coûteaux et al. 1995; Robinson 2002; Aerts 2006), the incubation treatment of +3°C did not emerge as a significant factor in our study. Perhaps a potentially stronger temperature effect would be observed on a longer time scale, because small differences in the rate of litter mass loss would have an increasingly visible effect on total litter mass remaining over time. The range of temperatures chosen in our experiment could also explain why temperature did not emerge as a significant main factor. The same absolute difference of 3°C can have very different relative effects when approaching threshold values for decomposer activity at the high or low end of the temperature gradient. With our selected range between 13 and 20°C, temperatures were not representing extreme, but rather average conditions. However, in the absence of *Glomeris*, we observed increased mass losses associated with the 3°C rise, in the monocultures of the two more rapidly decomposing species, *Tilia* and *Carpinus*. The more recalcitrant species, *Fagus* and *Quercus*, did not respond to temperature. This result does not support the general relationship between litter quality and temperature sensitivity of decomposition, which states that high-quality litter should be less sensitive to temperature than low-quality litter (Fierer et al. 2005). However, complex interactions between microbial decomposer communities, litter quality and temperature can occur in microcosms and lead to temperature responses that are more marked in litter materials of higher quality (Thormann et al. 2004).

It is interesting to stress that the few CO_2 and temperature effects were only observed when *Glomeris* was absent. This may indicate that microorganisms are more sensitive to shifts in substrate quality and temperature, because of immediate consequences on exoenzyme activity. In contrast, the feeding activity of macrofauna may be less responsive to such changes, at least within a certain range and outside critical thresholds. In particular, the two temperature treatments chosen in our experiment represent mild conditions for millipedes, and their consumption rates were probably not very different between 15 and 18°C (Coûteaux et al. 2002; David and Gillon 2002). This suggests that decomposition may be less affected by elevated CO_2 and temperature in ecosystems with an abundant macrofauna community, such as the studied temperate forest, than in ecosystems in which microbial processes predominate, such as some boreal forests. However, such short-term responses as observed in our experiment might change quickly through time.

The key importance of litter type and litter-feeding animals

The species composition of leaf litter had a considerable influence on both mass loss and mineralization in our microcosms. First, irrespective of the presence of *Glomeris*, there was a clear distinction between fast-decomposing species (*Tilia* and to a lesser degree *Carpinus*) and more

recalcitrant species (*Fagus* and *Quercus*). On average, both mass loss and C mineralization varied by a factor of 4.2 between *Tilia* and *Fagus* monocultures. Such species-related differences in decomposability are well documented and have often been correlated with various litter quality parameters (e.g. Cornelissen 1996; Pérez-Harguindeguy et al. 2000). In our study, N concentration and C:N ratio were the best predictors of decomposition rate.

Differences in litter quality among tree species had stronger effects on decomposition than the 3°C rise in temperature. This result is consistent with a recent large-scale analysis, showing that variation in litter mass loss among plant species is much greater than climate-driven variation (Cornwell et al. 2008). Also, differences in litter chemistry between species were more marked than CO_2-induced quality changes within species, which supports findings from previous studies (Cotrufo et al. 1998; Finzi et al. 2001; Norby et al. 2001). Collectively, these data show that the species composition and mixture of leaf litter has a greater potential to affect decomposition than elevated CO_2-induced quality changes or a moderate increase in temperature. Undoubtedly, changes in plant community composition and tree species range shifts need particular attention in evaluating the consequences of ongoing global change on decomposition and biogeochemical cycling. Our study simulated quite drastic differences in litter layer composition, whereas global change-induced shifts in plant community composition are likely to be both gradual and complex (Walther 2004; Midgley et al. 2007). Accordingly, the relative importance of biotic and abiotic components of global change effects on decomposition may depend on specific scenarios of plant community change.

While our results on single factor effects are largely in agreement with previous studies, we additionally show that a fully factorial analysis provides little evidence for interactive effects involving elevated CO_2 and temperature, at least over the short term. The only remarkable, highly significant interaction in our experiment was between litter type and presence of macrofauna. Although, overall, *Glomeris* consumption significantly increased litter mass loss in the microcosms, its effect varied greatly depending on the litter type. Macroarthropods generally show higher consumption rates on litter with low C:N ratios, once inhibitory factors such as secondary compounds or physical toughness are reduced during the initial stages of decomposition (Schaefer 1991; Wolters 2000; David et al. 2001). Thus, *Tilia* litter as the most favourable litter type for microbial decomposition, apparently was also the most palatable litter type for the millipede, resulting in the highest mass losses when *Glomeris* was associated with *Tilia*. The relative effect of *Glomeris* was actually stronger in some litter mixtures containing *Tilia* than in *Tilia* monocultures. This suggests that the *Glomeris* effect was not just driven by *Tilia* as the supposedly most palatable species included in our test, but to an important degree by the type of litter combination. Interestingly, the strongest effect of millipedes was observed when *Tilia* was mixed with *Fagus*, the two species representing the extremities of our litter quality gradient. The nutrient transfer hypothesis of litter mixture effects states that non-additive effects should particularly occur in mixtures of contrasting nutrient concentrations (Hättenschwiler et al. 2005), but empirical tests of this hypothesis have produced conflicting results (Hoorens et al. 2003; Schimel and Hättenschwiler 2007). In a recent paper, Pérez-Harguindeguy et al. (2008) found a greater positive difference between observed and expected decomposition rates in mixtures with higher heterogeneity in non-labile compounds (lignin, cellulose and hemicellulose), suggesting that chemical distance among litter species may be important for mixture effects (see also Epps et al. 2007).

In contrast to litter mass loss, C mineralization was not affected by the presence of *Glomeris*, which supports the findings of another experiment including various species of macrofauna feeding on *Fagus* litter (Hedde et al. 2007). This means that the largest part of litter material lost through fauna activity remained in the system and was not respired as CO_2. This suggests that the main effect of *Glomeris* was the physical transformation of litter material, with no immediately measurable effects on C mineralization. Such effects would be mainly indirect, by providing a more accessible substrate to microorganisms in the form of faeces. However, microorganisms apparently were not stimulated by millipede feeding activity over the duration of the experiment. In the *Carpinus*/*Tilia* mixture, there was even a reduction in C mineralization in the presence of *Glomeris*, suggesting an inhibiting fauna effect on microbial activity in this particular litter type. In fact, previous studies have shown that the impact of litter-feeding animals on organic matter mineralization is highly variable, depending on the age and quality of leaf litter (Maraun and Scheu 1996) and the animal species involved (Hedde et al. 2007). However, even if C mineralization initially remains unchanged, the conversion of vast amounts of leaf litter into faeces and their incorporation into the soil by the saprophagous macrofauna profoundly affect the spatio-temporal pattern of soil food web interactions (Wolters 2000; Lavelle and Spain 2001; Wardle 2002), which alters the fate of C and might affect C mineralization in the longer term.

The functional importance of the soil macrofauna clearly depends on their abundance and biomass. The *Glomeris* biomass in our microcosms corresponds to about 30 g live mass m^{-2}. This is about twice as high as the maximum millipede biomass recorded in Europe, but it is lower than the total biomass of saprophagous macrofauna

(earthworms, macroarthropods and gastropods) commonly found in mull-type forest soil, which can exceed 50–100 g live mass m^{-2} (Schaefer 1991; David 1999). So, while the total macrofauna biomass in our study is considered to reflect field conditions quite realistically, the dominance of only one species is certainly not typical. A more diverse macrofauna community would probably modify our results, because different species of litter-feeding invertebrates have been shown to affect litter decomposition distinctly (Hättenschwiler and Gasser 2005; Hedde et al. 2007) and because multiple species may interact in non-additive ways (Heemsbergen et al. 2004; Zimmer et al. 2005).

Conclusion

We acknowledge that our experimental approach of using artificial microcosms over a short time period may be limited for the scaling up to real ecosystems. However, the spatial scale (centimetres) at which the studied processes occur in the field is not very different from the microcosm situation. Our data have shown that substantial changes in the species composition of tree leaf litter have stronger effects on litter mass loss and C mineralization than CO_2-induced litter quality changes and a 3°C rise in temperature during decomposition. In addition, the effects of litter species composition on litter mass loss are substantially modified when saprophagous macrofauna are present in the system. These results suggest that biotic factors are of key importance for predicting global change effects on decomposition and nutrient cycling in temperate forest ecosystems. Depending on the degree of global change-induced shifts in the composition of plant and soil invertebrate communities, these changes in biodiversity may have more important consequences than the direct effects of abiotic factors on litter chemistry and reaction kinetics.

Acknowledgements We thank Benjamin Jackson for his contributions to the set-up of the experiment, animal collections in the field, laboratory support and discussions, Bruno Buatois, Laurette Sonié, Raphaëlle Leclerc, Sandrine Bioulac, Sylvain Coq and Jérémy Devaux for laboratory assistance, Christian Collin and David Delguedre for technical support, Erwin Amstutz, Markus Schindler and Mark Gessner for their help in litter collections, Christian Körner for his support of work at the Swiss Canopy Crane (SCC) research site in Basel, and anonymous reviewers for thoughtful comments that improved previous versions of our manuscript. This research was possible thanks to the support of the SCC research site and the CO_2 enrichment facility by the Swiss National Science Foundation (grants 3100-0597769.99 and 3100-067775.02), the Swiss Federal Office for the Environment (BUWAL) and the University of Basel. Funding was provided by the ESF EUROCORES programme EuroDIVERSITY to the collaborative research project BioCycle. BioCycle is endorsed by DIVERSITAS as contributing towards their current scientific research priorities in biodiversity science.

References

Aerts R (2006) The freezer defrosting: global warming and litter decomposition rates in cold biomes. J Ecol 94:713–724

Bardgett RD (2005) The biology of soil. A community and ecosystem approach. Oxford University Press, New York

Bradley KL, Pregitzer KS (2007) Ecosystem assembly and terrestrial carbon balance under elevated CO_2. Trends Ecol Evol 22:538–547

Chapin FS III, Matson PA, Mooney HA (2002) Principles of terrestrial ecosystem ecology. Springer, New York

Chung H, Zak DR, Lilleskov EA (2006) Fungal community composition and metabolism under elevated CO_2 and O_3. Oecologia 147:143–154

Chung H, Zak DR, Reich PB, Ellsworth DS (2007) Plant species richness, elevated CO_2 and atmospheric nitrogen deposition alter soil microbial community composition and function. Global Change Biol 13:980–989

Cornelissen JHC (1996) An experimental comparison of leaf decomposition rates in a wide range of temperate plant species and types. J Ecol 84:573–582

Cornwell WK, Cornelissen JHC, Amatangelo K, et al. (2008) Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecol Lett 11:1065–1071

Cotrufo MF, Ineson P (1996) Elevated CO_2 reduces field decomposition rates of *Betula pendula* Roth. leaf litter. Oecologia 106:525–530

Cotrufo MF, Briones MJI, Ineson P (1998) Elevated CO_2 affects field decomposition rate and palatability of tree leaf litter: importance of changes in substrate quality. Soil Biol Biochem 30:1565–1571

Cotrufo MF, De Angelis P, Polle A (2005) Leaf litter production and decomposition in a poplar short-rotation coppice exposed to free air CO_2 enrichment (POPFACE). Global Change Biol 11:971–982

Coûteaux MM, Mousseau M, Célérier ML, Bottner P (1991) Increased atmospheric CO_2 and litter quality: decomposition of sweet chestnut leaf litter with animal food webs of different complexities. Oikos 61:54–64

Coûteaux MM, Bottner P, Berg B (1995) Litter decomposition, climate and litter quality. Trends Ecol Evol 10:63–66

Coûteaux MM, Aloui A, Kurz-Besson C (2002) *Pinus halepensis* litter decomposition in laboratory microcosms as influenced by temperature and a millipede, *Glomeris marginata*. Appl Soil Ecol 20:85–96

David JF (1999) Abundance, biomass and functional structure of the saprophagous macrofauna in the litter and soil of Mediterranean oak forests. Pedobiologia 43:319–327

David JF, Gillon D (2002) Annual feeding rate of the millipede *Glomeris marginata* on holm oak (*Quercus ilex*) leaf litter under Mediterranean conditions. Pedobiologia 46:42–52

David JF, Gillon D (2009) Combined effects of elevated temperatures and reduced leaf litter quality on the life-history parameters of a saprophagous macroarthropod. Global Change Biol 15:156–165

David JF, Malet N, Coûteaux MM, Roy J (2001) Feeding rates of the woodlouse *Armadillidium vulgare* on herb litters produced at two levels of atmospheric CO_2. Oecologia 127:343–349

Epps KY, Comerford NB, Reeves JB, Cropper WP, Araujo QR (2007) Chemical diversity—highlighting a species richness and ecosystem function disconnect. Oikos 116:1831–1840

Fierer N, Craine JM, McLauchlan K, Schimel JP (2005) Litter quality and the temperature sensitivity of decomposition. Ecology 86:320–326

Finzi AC, Allen AS, DeLucia EH, Ellsworth DS, Schlesinger WH (2001) Forest litter production, chemistry and decomposition

following two years of free air CO_2 enrichment. Ecology 82:470–484
Gartner TB, Cardon ZG (2004) Decomposition dynamics in mixed-species leaf litter. Oikos 104:230–246
Hagedorn F, Machwitz M (2007) Controls on dissolved organic matter leaching from forest litter grown under elevated CO_2. Soil Biol Biochem 39:1759–1769
Hättenschwiler S, Gasser P (2005) Soil animals alter plant litter diversity effects on decomposition. Proc Natl Acad Sci USA 102:1519–1524
Hättenschwiler S, Bühler S, Körner C (1999) Quality, decomposition and isopod consumption of tree litter produced under elevated CO_2. Oikos 85:271–281
Hättenschwiler S, Tiunov AV, Scheu S (2005) Biodiversity and litter decomposition in terrestrial ecosystems. Annu Rev Ecol Evol Syst 36:191–218
Heal OW, Anderson JM, Swift MJ (1997) Plant litter quality and decomposition: an historical overview. In: Cadisch G, Giller KE (eds) Driven by nature. CAB International, Wallingford, pp 3–30
Hector A, Bagchi R (2007) Biodiversity and ecosystem multifunctionality. Nature 448:188–190
Hedde M, Bureau F, Akpa-Vinceslas M, Aubert M, Decaëns T (2007) Beech leaf degradation in laboratory experiments: effects of eight detritivorous invertebrate species. Appl Soil Ecol 35:291–301
Heemsbergen DA, Berg MP, Loreau M, Van Hal JR, Faber JH, Verhoef HA (2004) Biodiversity effects on soil processes explained by interspecific functional dissimilarity. Science 306:1019–1020
Hoorens B, Aerts R, Stroetenga M (2003) Does initial litter chemistry explain litter mixture effects on decomposition? Oecologia 137:578–586
Hyvönen R, Agren GI, Linder S, et al. (2007) The likely impact of elevated [CO_2], nitrogen deposition, increased temperature and management on carbon sequestration in temperate and boreal forest ecosystems: a literature review. New Phytol 173:463–480
IPCC (2007) Climate change 2007: the physical science basis. In: Solomon S, Qin D, Manning M, et al. (eds) Contribution of Working Group I to the fourth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge
Körner C (2000) Biosphere responses to CO_2 enrichment. Ecol Appl 10:1590–1619
Körner C, Asshoff R, Bignucolo O, et al. (2005) Carbon flux and growth in mature deciduous forest trees exposed to elevated CO_2. Science 309:1360–1362
Lavelle P, Spain AV (2001) Soil ecology. Kluwer, London
Loranger G, Ponge JF, Imbert D, Lavelle P (2002) Leaf decomposition in two semi-evergreen tropical forests: influence of litter quality. Biol Fertil Soils 35:247–252
Loreau M, Naeem S, Inchausti P, et al. (2001) Biodiversity and ecosystem functioning: current knowledge and future challenges. Science 294:804–808
Maraun M, Scheu S (1996) Changes in microbial biomass, respiration and nutrient status of beech (*Fagus sylvatica*) leaf litter processed by millipedes (*Glomeris marginata*). Oecologia 107:131–140
Midgley GF, Thuiller W, Higgins SI (2007) Plant species migration as a key uncertainty in predicting future impacts of climate change on ecosystems: progress and challenges. In: Canadell JG, Pataki DE, Pitelka LF (eds) Terrestrial ecosystems in a changing world. Springer, Berlin, pp 129–137
Norby RJ, Cotrufo MF, Ineson P, O'Neill EG, Canadell JG (2001) Elevated CO_2, litter chemistry, and decomposition: a synthesis. Oecologia 127:153–165
Parsons WJF, Bockheim JG, Lindroth RL (2008) Independent, interactive and species-specific responses of leaf litter decomposition to elevated CO_2 and O_3 in a northern hardwood forest. Ecosystems 11:505–519
Pendall E, Bridgham S, Hanson PJ, et al. (2004) Below-ground process responses to elevated CO_2 and temperature: a discussion of observations, measurement methods, and models. New Phytol 162:311–322
Pepin S, Körner C (2002) Web-FACE: a new canopy free-air CO_2 enrichment system for tall trees in mature forests. Oecologia 133:1–9
Pérez-Harguindeguy N, Díaz S, Cornelissen JHC, Vendramini F, Cabido M, Castellanos A (2000) Chemistry and toughness predict leaf litter decomposition rates over a wide spectrum of functional types and taxa in central Argentina. Plant Soil 218:21–30
Pérez-Harguindeguy N, Blundo CM, Gurvich DE, Díaz S, Cuevas E (2008) More than the sum of its parts? Assessing litter heterogeneity effects on the decomposition of litter mixtures through leaf chemistry. Plant Soil 303:151–159
Robinson CH (2002) Controls on decomposition and soil nitrogen availability at high latitudes. Plant Soil 242:65–81
Sala OE, Chapin FS, Armesto JJ, et al. (2000) Global biodiversity scenarios for the year 2100. Science 287:1770–17774
Saugier B, Roy J, Mooney HA (2001) Estimation of global terrestrial productivity: converging toward a single number? In: Roy J, Saugier B, Mooney HA (eds) Terrestrial global productivity. Academic Press, London, pp 543–557
Sayer EJ (2006) Using experimental manipulation to assess the roles of leaf litter in the functioning of forest ecosystems. Biol Rev 81:1–31
Schaefer M (1991) Secondary production and decomposition. In: Röhrig E, Ulrich B (eds) Ecosystems of the world 7. Temperate deciduous forests. Elsevier, Amsterdam, pp 175–218
Schimel JP, Hättenschwiler S (2007) Nitrogen transfer between decomposing leaves of different N status. Soil Biol Biochem 39:1428–1436
R Development Core Team (2005) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. ISBN 3-900051-07-0. http://www.R-project.org
Thormann MN, Bayley SE, Currah RS (2004) Microcosm tests of the effects of temperature and microbial species number on the decomposition of *Carex aquatilis* and *Sphagnum fuscum* litter from southern boreal peatlands. Can J Microbiol 50:793–802
Wall DH, Bradford MA, St. John MG, et al. (2008) Global decomposition experiment shows soil animal impacts on decomposition are climate-dependent. Global Change Biol 14:2661–2677
Walther GR (2004) Plants in a warmer world. Perspectives in plant ecology evolution and systematics 6:169–185
Wan S, Norby RJ, Ledford J, Weltzin JF (2007) Responses of soil respiration to elevated CO_2, air warming, and changing soil water availability in a model old-field grassland. Global Change Biol 13:2411–2424
Wardle D (2002) Communities and ecosystems. Linking the aboveground and belowground components. Princeton University Press, Princeton
Wolters V (2000) Invertebrate control of soil organic matter stability. Biol Fertil Soils 31:1–19
Zimmer M, Kautz G, Topp W (2005) Do woodlice and earthworms interact synergistically in leaf litter decomposition? Funct Ecol 19:7–16