Soil quality, leaf litter quality, and microbial biomass interactively drive soil respiration in a microcosm experiment

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Abstract

Soil respiration plays a central role in global carbon dynamics, and small changes in the magnitude of soil respiration could have large impacts on atmospheric CO_2 concentrations. Heterotrophic soil respiration mainly comes from microbial mineralization of soil organic matter and decomposition of plant litter, yet only a few studies have addressed the combined effects of interactions among leaf litter quality, soil quality, and microbial biomass on soil respiration. We conducted a microcosm experiment using three soils from three forest sites representing a gradient in soil quality, comprised of soil pH and C:N ratio, and six tree litter types (from the same forests), encompassing a gradient in leaf nutrient and lignin concentrations. We followed soil CO_2 emissions, soil basal respiration (measured as O_2-consumption), and microbial biomass over twelve weeks to examine variation in response to leaf litter and soil quality and their interactions. Our results show that soil CO_2 emissions increased significantly with soil quality and leaf litter quality respectively, and these effects were mediated by interactions with soil microbial biomass. Moreover, we found idiosyncratic interactive effects of leaf litter quality and microbial biomass on soil CO_2 emissions across the gradient in soil quality. The sensitivity of soil respiration to soil quality and the interactions between leaf litter quality and soil microbial biomass suggests that global change drivers altering forest composition and soil community composition may have significant cascading effects on the soil carbon cycle.

Keywords Aboveground-belowground interactions | carbon cycle | decomposition | leaf litter C dynamics | leaf litter traits | context-dependency

1. Introduction

Soil respiration is one of the largest CO_2 fluxes in the global carbon cycle, and small changes in the magnitude of soil respiration could have a significant effect on atmospheric CO_2 concentrations (Schuur et al. 2015, Subke et al. 2006). Soil respiration usually consists of autotrophic and heterotrophic respiration (Schlesinger & Andrews 2000, Luo & Zhou 2006), the latter of which comes from microbial litter decomposition and mineralization of soil organic matter (Hanson et al. 2000, Kuzyakov 2006, Zhou et al. 2014). Previous studies have shown that factors controlling heterotrophic soil respiration and determining its temporal and spatial variation at large spatial scales (i.e., kilometers or larger) are mostly related to abiotic factors (e.g., soil water content and temperature) (Bauer

et al. 2008, Manzoni et al. 2012a, Moyano et al. 2013, Yan et al. 2018), and their impacts on soil respiration is well understood for a variety of forest ecosystems (Bonal et al. 2008, Adachi et al. 2009). However, at smaller spatial scales (i.e., meters or less), soil respiration appears to be related to biotic (e.g., microbial activity) rather than abiotic factors (Scott-Denton et al. 2003, Katayama et al. 2009, Martin & Bolstad 2009, Fanin et al. 2011), although the impacts of biotic factors on soil respiration are not certain (Hooper et al. 2000).

Microbial biomass can be a sensitive indicator of changes in soil organic matter (Kuzyakov 2010), as it is involved in organic matter mineralization (Nannipieri et al. 2002), and thus influences soil respiration. For instance, Denton et al. (2003) showed that soil microbial biomass carbon (C) is positively correlated with soil respiration rate. Anderson & Domsch (1993) found that total soil microbial biomass and microbial respiration rates were lower in acidic soils than in soils with neutral pH. Moreover, previous studies have shown that higher soil N availability and lower soil C availability inhibited microbial biomass and microbial respiration (Demoling et al. 2008, Tian et al. 2018). Thus, 'high-quality' soil, e.g., soil with high soil C concentration and neutral soil pH, would likely induce high microbial activity and high soil respiration, while 'low-quality' soil with low soil C concentration and acid soil pH would likely induce low soil microbial activity and low soil respiration (Huang et al. 2021, Xu 2018).

'High-quality' leaf litter, e.g., leaf litter with low C:N and lignin concentrations and high concentrations of N, P, K, Ca, and Mg (Cornwell et al. 2008, Zhang et al. 2008, Hobbie et al. 2010), decomposes quickly, while 'low-quality' leaf litter decomposes comparatively slowly (Freschet et al. 2012, Cotrufo et al. 2013, Santos & Balieiro 2018). For instance, Cleveland et al. (2014) showed that changes in leaf litter quality explained the majority of variation in decomposition rates under controlled laboratory conditions. However, leaf litter decomposition rates are typically estimated via measurements of litter mass loss, which do not account for how much is partitioned to respiration and decomposer biomass (Rubino et al. 2010, 2007). Carbon from decomposing organic matter can either be used for the growth of new cells (microbial biomass production) or respired for energy production (CO_2 emissions) (Six et al. 2006, Sinsabaugh et al. 2013). When microbial C-use efficiency, i.e., the ratio of decomposer growth rate to the rate of organic matter uptake, is high, more litter-derived C is converted to biomass and less litter-derived C is respired to CO_2 (Manzoni et al. 2012b). Because leaf litter quality regulates the proportion of easily assimilated C compounds to soil microbes, increasing leaf litter lignin concentration decreases microbial C-use efficiency (Lekkerkerk et al.

1990). This results in a greater allocation of leaf litter-derived C towards microbial biomass production than to CO_2 emissions with high leaf litter quality (Cotrufo et al. 2013). Thus, positive effects of leaf litter quality on leaf litter mass loss rates and microbial C use efficiency may have opposing effects on soil CO_2 emissions (Mueller et al. 2015).

Leaf litter quality and microbial biomass jointly influence microbial C-use efficiency (Stewart et al. 2015, Manzoni 2012b). Soil microorganisms are primarily C-limited, implying that the growth of lower microbial biomass requires less organic C to meet C requirements in comparison to high microbial biomass (Farrell et al. 2014). This suggests that the amount of leaf litter-derived C allocated to microbial biomass or CO_2 is not uniquely dependent on leaf litter quality (Mueller et al. 2015). However, the contributions of the interactive effects of leaf litter quality and microbial biomass on soil respiration are unclear, particularly across gradients in soil quality (Bradford et al. 2014). Moreover, the extent to which soil quality mediates the effect of plant-microbe interactions on soil respiration is an open question with important implications for soil ecosystem processes like soil carbon storage and nutrient retention (Lange et al. 2015, Leimer et al. 2016). While the spatial overlap of gradients in soil and leaf litter quality in real-world ecosystems is increasingly recognized (Bardgett & Putten 2014, Mueller et al. 2015), the impacts of interactions between soil quality and leaf litter quality on soil respiration are less well understood.

In the present study, we examined the extent to which (a) variation in soil quality and (b) variation in leaf litter quality drive variation in soil respiration, and (c) interactions among soil quality, leaf litter quality, and microbial biomass influence soil respiration. We conducted a microcosm experiment for which we used three forest soils that varied in C:N and pH, representing a gradient in soil quality, and leaf litter from six deciduous tree species, representing a gradient in litter quality. We assessed the response of soil respiration (soil CO_2 emissions in conjunction with litter) and soil microbial properties (basal respiration of soil only, microbial biomass) over twelve weeks to variation in soil and litter quality as well as their interaction. We hypothesized that: (1) soil respiration would significantly increase with increasing soil quality due to high soil carbon and nitrogen (N) availability and soil microbial biomass; (2) soil respiration would increase significantly with increasing leaf litter quality due to faster decomposition rates of high-quality leaf litter (Kuzyakov 2010, Cotrufo et al. 2013); and (3) the slope of the relationship between soil respiration and microbial biomass would strengthen with increasing leaf litter quality (Cotrufo et al. 2013, Farrell et al. 2014, Manzoni 2012b).

2. Materials and methods
2.1 Experimental setup

To investigate the interaction effects of soil quality and leaf litter quality on soil microorganisms, we established a microcosm experiment with five replicates for each treatment (3 soil quality and 6 leaf litter quality). We used microcosms made of polyvinyl chloride (PVC) tubes (inner diameter 6.2 cm, height 15 cm), which were placed on ceramic plates to allow water drainage. At the top edge, each microcosm had two milled grooves, into each of which a sealing rubber was fitted. This helped to seal the lid airtight, which is important for following measurements of CO_2 emission. Therefore, each lid had a small additional container for the CO_2 trap (more details about measuring CO_2 emission below). This experimental set-up has proven successful for studying soil and leaf litter quality effects on soil microorganisms (Cesarz et al. 2016). Microcosms were incubated for one week after which one earthworm (Aporrectodea caliginosa; the starting mass was 0.164 ± 0.087 g) was added to mimic natural soil conditions, given their importance in organic matter turnover and nutrient cycling in European temperate forests (Blouin et al. 2013). More generally, earthworms are an important component of terrestrial ecosystems (Phillips et al. 2019) that should not be ignored in experiments. While earthworms may contribute directly to total soil respiration, we expected that the main contribution of earthworms to soil respiration would be indirect, i.e. by increasing microbial activity or litter decomposition rates. In total, the experiment was run for a period of 12 weeks. We recognize that considerably more time would be needed for litter to fully decompose, but in this study we focused on the effects of early stage decomposition on soil respiration and soil microbial properties, which are widely studied (Djukic et al. 2018, Djukic et al. 2021, Kwon et al. 2021).

Each microcosm was filled with 210 g of air-dried soil. Soils varying in C:N and pH were taken from old-growth deciduous forests located in the north-eastern part of the Hainich National Park (51°04′N, 10°30′E) in central Germany at an elevation of about 350 m a.s.l. The forest stands differ in tree species diversity, with site 1a (low soil quality) having the lowest Shannon diversity for tree genera (0.2±0.1), site 2a (medium soil quality) having medium Shannon diversity for tree genera (1.0±0.1), and site 3a (high soil quality) having the highest Shannon diversity for tree genera (1.4±0.0). Sites have a minimum distance of ca. 440 m and a maximum distance of ca. 1730 m (Jacob et al. 2009, Mölder et al. 2006). The mean annual temperature is 7.5°C, and the mean annual precipitation ranges from 600 mm to 670 mm (Jacob et al. 2009). Differences in soil quality, e.g., soil C:N and pH (Tab. S1), resulted from differences in the proportions of beech (Fagus sylvatica L.) (Leuschner et al. 2009). The three forest types were (1) a site dominated by 94 % beech (low soil quality), (2) a site with 70 % beech as well as lime (Tilia cordata Mill. and T. platyphyllos Scop.), and ash (F. excelsior L.) (medium soil quality), and (3) a site with a low proportion of beech (7 %) and high proportions of lime, ash, hornbeam (Carpinus betulus L.), Sycamore maple (Acer pseudoplatanus L.), and Norway maple (Acer platanoides L.) (high soil quality). In August 2006, the soil was collected from the upper soil layer (to a depth of 5 cm) of the three forest types. The soil was sieved with a 4 mm mesh to remove animals, roots, and plants, defaunated by oven-drying at 55°C for seven days and then stored at room temperature. Drying at 55°C is lethal for soil meso-and macrofauna, but not for soil microbes (Huhta et al. 1989). After microcosms were filled with air-dried soil, they were watered initially with 216 ml deionized water to mimic the mean natural soil water content at the study site. All microcosms were placed in a climate chamber at 15°C day and night without light.

Six different leaf litter types representing the dominant tree species in the sampled forests and a gradient in leaf litter quality (from low to high; Fig. 1, Tab. S2) (Cesarz et al. 2016, Jacob et al. 2010b) were collected from the mixed-species forest: beech, Norway maple, sycamore maple, hornbeam, lime, and ash. Leaf litter was air-dried and stored at room temperature. Here, we define leaf litter quality based on leaf litter C, P, N, Ca, and Mg concentrations, C:N, C:P, and N:P ratios, and lignin content, given the importance of these leaf litter traits for decomposition (e.g., Zhang et al. 2008, Makkonen et al. 2012, Cesarz et al. 2016).

The C and N concentrations of the leaf litter were measured using an automated C:N analyser (Heraeus Elementar Vario EL, Hanau, Germany). All other following nutrients were retrieved from a study of the same study site (Jacob et al. 2009, 2010b, Thoms et al. 2010). The concentrations of Ca, P, and Mg in the leaf litter were determined by ICP-AES (Spectro, Kleve, Germany) after pressure digestion with concentrated nitric acid. The bulk of the samples was analyzed for lignin concentration using near-infrared spectroscopy (NIRS) as a rapid and accurate method for determining lignin in a large number of samples (Ono et al. 2008). Every microcosm received 504 mg carbon in the form of leaf litter. For low quality soil, this amounted to 7.3 g C kg⁻¹ dry soil, 6.3 g C kg⁻¹ dry soil for medium quality soil, and 6.2 g C kg⁻¹ dry soil for high quality soil (Cesarz et al. 2016). Litter was cut into small pieces (2 x 2 cm) and added to microcosms at the surface of soils.

2.2   Sampling and measurements

Every three weeks, we measured soil CO_2 emission, soil basal respiration, and microbial biomass of each microcosm over a period of three months (four measurements in total). During the experiment, microcosms were watered bi-weekly with 72 ml deionized water to prevent soil desiccation. For soil CO_2 emissions, we sealed microcosms for 24 h and used 2 ml 1N KOH to absorb total soil CO_2 emissions (Kuzyakov et al. 2009, Marhan et al. 2007). The consumed amounts of KOH by CO_2 (ml day^{-1}) were measured by titration of 1M HCL. At the same time, soil basal respiration (i.e., mineralization of soil organic matter without litter effects) and microbial biomass were measured using an O^2-microcompensation device (Substrate-induced respiration, SIR; Anderson & Domsch 1978, Scheu 1992). We took 5 g fresh soil from each microcosm and measured basal respiration, i.e., respiration without adding any substrate, at hourly intervals for 24 h at 22°C (BAS: µl O^2 h^{-1} g^{-1} soil dry weight). The experiment was run at 15°C, whereas the SIR measurements were run at 20°C. The mean of the last 10 h was used to calculate basal respiration. Here, only a fraction of the total microbial community is active. Afterwards, D-glucose was added to the same soil to measure microbial biomass C. Glucose was added to saturate the catabolic enzymes of the microorganisms (4 mg g-1 dry mass solved in 400 µl deionized water), which activated a large proportion of the microbial community. The mean of the lowest three readings within the first 3 h, i.e., before microorganisms started to grow, was taken as the maximum initial respiratory response (MIRR: µl O^2 h^{-1} g^{-1} soil dry weight) and microbial biomass

(Cmic: µg Cg^{-1} soil dry weight) was calculated as 38×MIRR (Beck et al. 1997). Although we did not measure microbial properties at the beginning of the experiment, initial values likely captured differences in soil quality associated with soil organic matter (Kuzyakov 2010), which persisted throughout the experiment because leaf litter typically does not affect soil organic carbon content over short periods of time (Fang et al 2015).

2.3   Data analysis

We performed principal component analysis (PCA) separately on characteristics related to soil and leaf litter quality (Fig. 1) and used species scores from the first principal components axis as continuous predictor variables for both because the first axis explained the most variation for soil quality (89.6%) and leaf litter quality (62.0%). We standardized the scores of soil quality and leaf litter quality from the PCA analysis using the scale function in the 'base' R package. We fitted linear mixed effects models using the 'nlme' package to analyze variation in soil CO_2 emissions, basal respiration, and microbial biomass. Soil quality, leaf litter quality, time, and their interactions were treated as fixed effects. Random effects were included for microcosm identity, and we accounted for repeated measurements at the microcosm level by using a compound symmetric covariance structure. Model assumptions were assessed visually by inspecting residual plots for homogeneity and quantile-quantile plots for normality. To test for the effects of leaf litter–microbe interactions on soil CO_2 emissions, we fitted separate linear mixed-effects models for each
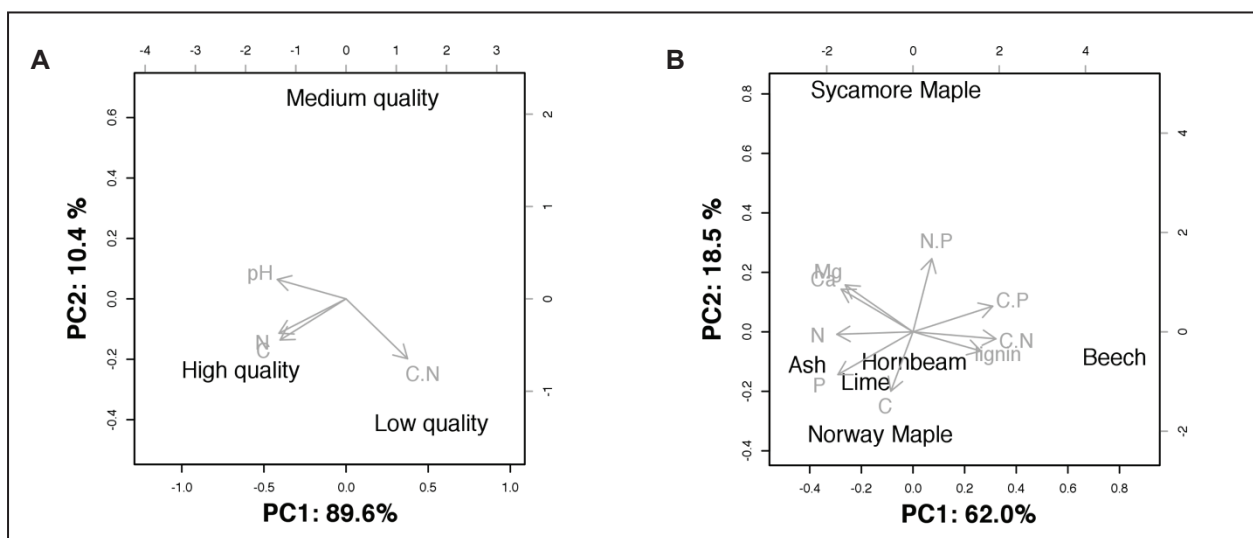

Figure 1. Principal components analysis of (**A**) soil quality and (**B**) leaf litter quality across the experimental treatments (Table S1-2). Soil quality was quantified as a combination of soil pH, C, N, and C:N; leaf litter quality was quantified as a combination of leaf Ca, C, lignin, Mg, N, P, C:N, C:P, and N:P. Soil and leaf litter were collected from Hainich National Park, Germany.

level of soil quality (low, medium, and high), where leaf litter quality, microbial biomass (Cmic), time, and their interactions were fixed effects. Random effects and covariance structures were modeled as described above. All analyses were performed using R software version 3.1.1 (The R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1 Soil quality and leaf litter quality effects on soil CO_2 emissions

Soil CO_2 emissions increased significantly with soil quality, leaf litter quality, and decreased significantly with time (Tab. 1, Fig. 2). Additionally, the high soil and leaf litter quality effects on soil CO_2 emissions decreased more rapidly over time than those of low soil and leaf litter quality (Fig. 2, Tab. 1). Basal respiration

increased significantly with increasing soil quality and decreased significantly with time (Tab. 1, Fig. 3A). On average, microbial biomass was higher in higher quality soil, although not initially due to small differences in soil nutrient status between high and medium quality soils (Tab. 1, Fig. 3B). Leaf litter quality did not show statistically significant effects on basal respiration and microbial biomass (Tab. 1). The interactions between soil quality and leaf litter quality did not significantly influence soil respiration (Tab. 1).

3.2 Interactive effects of leaf litter quality and microbial biomass on soil respiration

For each level of soil quality, we observed significant interactive effects of leaf litter quality and microbial biomass on soil CO_2 emissions (Tab. 2). With increasing soil microbial biomass, the response of soil CO_2 emissions
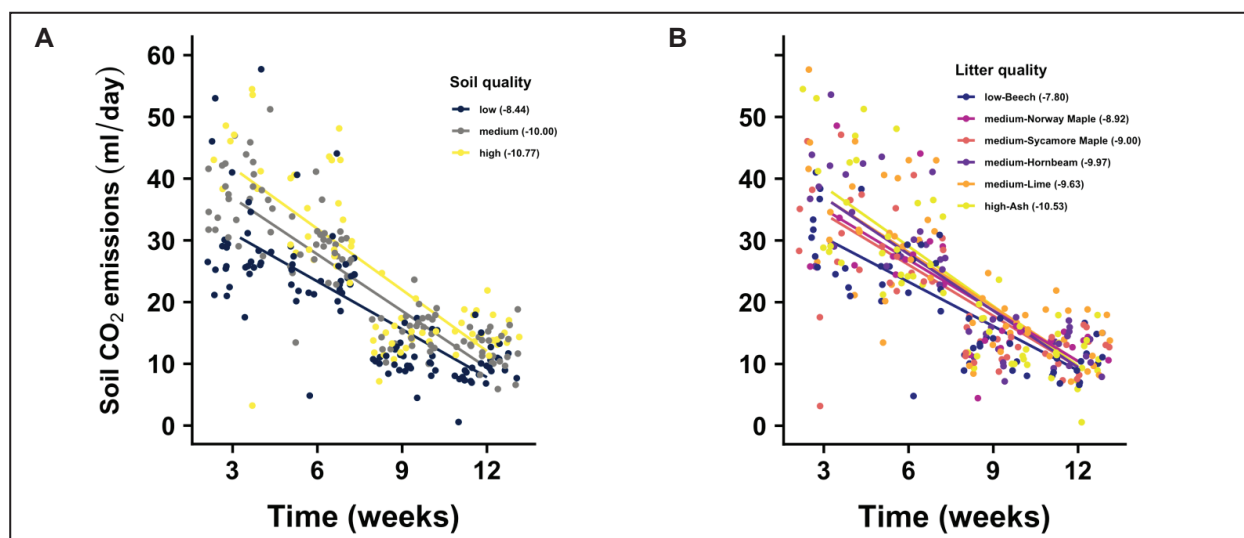

Figure 2. Responses of soil CO2 emissions to variations in (**A**) soil quality and (**B**) leaf litter quality over time. Lines represent each level of (**A**) soil quality and (**B**) leaf litter quality and were fitted using linear mixed-effects models (Table 1). The numbers in the brackets are estimated slopes for each line. Points are jittered to improve visualization. Soil quality is a continuous variable representing a gradient in soil C and N concentrations, pH, and C:N. Leaf litter quality is a continuous variable representing a gradient in litter C, N, P, Ca, Mg, and lignin concentrations, C:N, C:P, and N:P ratio.

Table 1. Summary of linear mixed-effects models examining variation in soil CO_2 emissions in response to soil quality (SQ), leaf litter quality (LQ), (T), and their interactions.

Fixed effects	Soil CO_2 emissions			Basal respiration			Microbial biomass		
	df	F	P-value	df	F	*P-value*	df	F	*P-value*
SQ	1,72	25.80	**0.00**	1,72	10.46	**0.00**	1,71	135.60	0.00
LQ	1,72	5.24	**0.03**	1,72	1.04	0.31	1,71	0.60	0.44
T	1,208	256.71	**0.00**	1,174	28.90	**0.00**	1,136	2.04	0.16
SQ:LQ	1,72	0.02	0.90	1,72	0.83	0.37	1,71	0.25	0.62
SQ:T	1,208	5.22	**0.02**	1,174	2.61	0.11	1,136	0.49	0.49
LQ:T	1,208	3.82	0.05	1,174	0.40	0.52	1,136	0.06	0.81
SQ:LQ:T	1,208	0.00	0.97	1,174	2.48	0.12	1,136	3.29	0.07

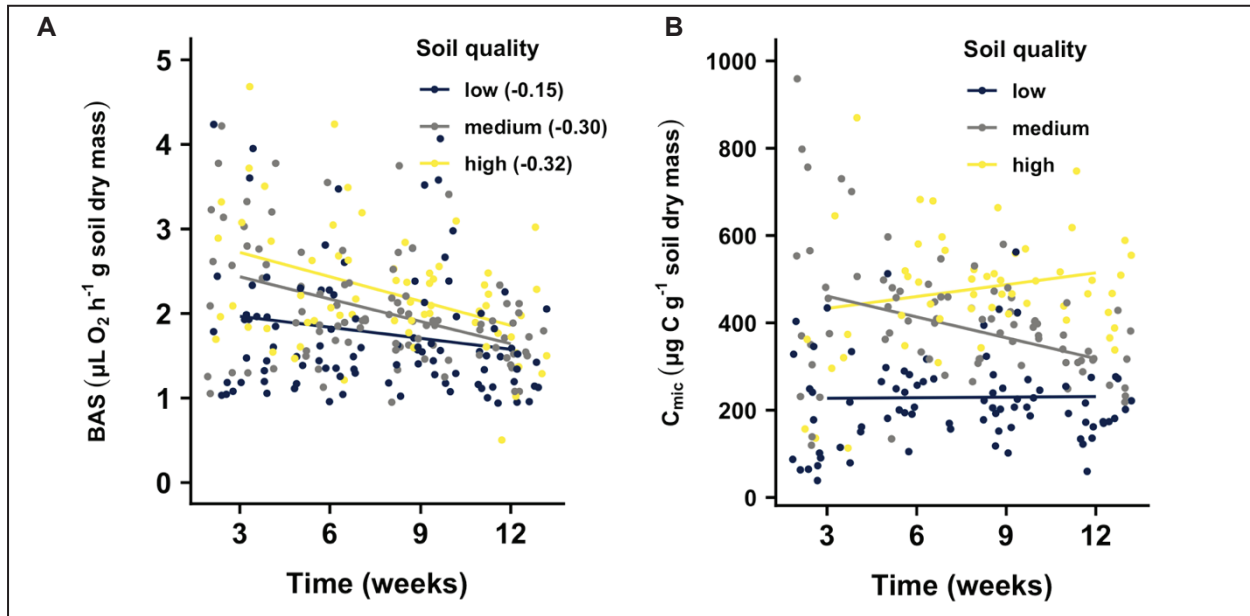Notes: Significant differences (*P*-value < 0.05) are displayed in bold.

Figure 3. Responses of (**A**) soil basal respiration (BAS), (**B**) soil microbial biomass (Cmic) to variations in soil quality over time. Lines are predicted using linear mixed-effects models (Table 1). The numbers in the brackets are estimated slopes for each line. Points are jittered to improve visualisation. Soil quality is a continuous variable representing a gradient in soil C and N concentrations, pH, and C:N (Fig. 1A).

Table 2. Summary of linear mixed-effects models evaluating relationships between soil CO_2 emissions, microbial biomass (Cmic), leaf litter quality (LQ), time (T) and their interactions in different quality soils.

Fixed effects	Low quality soil			Medium quality soil			High quality soil		
	df	F	P-value	df	F	P-value	df	F	P-value
LQ	5,71	2.67	**0.03**	5,67	7.04	0.00	5,34	1.71	0.16
T	1,71	191.62	**0.00**	1,67	430.33	**0.00**	1,34	92.77	**0.00**
Cmic	1,71	0.01	0.93	1,67	1.68	0.20	1,34	0.05	0.83
LQ:T	5,71	1.39	0.24	5,67	1.35	0.26	5,34	1.31	0.28
LQ:Cmic	5,71	3.05	**0.02**	5,67	2.94	**0.02**	5,34	3.75	**0.00**
T:Cmic	1,71	1.90	0.17	1,67	13.34	**0.00**	1,34	1.31	0.26
LQ:T:Cmic	5,71	2.25	0.06	5,67	1.06	0.39	5,34	2.73	**0.03**

Notes: Significant differences (*P*-value < 0.05) are displayed in boldface. LQ = litter quality, T = time.

to leaf litter quality was highly variable, which was contrary to our prediction that soil CO_2 emissions would increase with leaf litter quality (Fig. 4).

4. Discussion
4.1 Soil quality effects on soil CO_2 emissions via influencing soil microorganisms

We found that soil CO_2 emissions increased significantly across a soil quality gradient (Tab. 1, Fig. 2A). Without considering leaf litter quality effects, the variation in the response of soil CO_2 emissions to soil quality was mediated by the mineralization of soil organic matter, i.e., basal respiration and microbial biomass. Soil carbon and nutrient availability are particularly important for soil

microorganisms, indirectly influencing soil respiration (Kuzyakov 2006, Zhang et al. 2018). Moreover, previous studies have recognized the influence of soil C and N on soil microbial biomass (Demoling et al. 2008). For instance, Tian et al. (2017) found a positive relationship between microbial biomass and soil C content. Similarly, soil pH has also been recognized as a critical factor moderating the activity of microbial communities, as soil bacteria are inhibited when soil pH is below 4.5 (Högberg et al. 2007, Rousk et al. 2011, Chen et al. 2013). In our study, high soil quality, which was associated with high soil C (3.9 %), N (0.31 %), and pH (5.2) and low C:N(12.6), resulted in the positive relationships between soil quality and basal respiration and microbial biomass. We found positive relationships between soil basal respiration, microbial biomass, and soil quality (Tab. 1, Fig 3), which is consistent with previous studies showing that high soil quality (high soil C/N, low soil pH) supported high levels

of soil microbial biomass and basal respiration (Sjöberg et al., 2003, Bowden et al. 2004, Knorr et al. 2005, Demoling et al. 2008). As a result, increased basal respiration and microbial biomass increased soil CO_2 emission in soils with high quality. Our finding that soil CO_2 emissions, basal respiration, and microbial biomass were highest in high quality soil from a diverse forest and lowest in low quality soil from a forest dominated by beech (Fig. 2A), potentially supports the idea that plant community composition (Laganière et al. 2012) and/or plant species richness can alter soil ecosystem processes (Reich et al. 2005, Zhou et al. 2006, Eisenhauer et al. 2010, Huang et al. 2011, Muller et al. 2012, Thakur et al. 2015, Zhou et al, 2019, Xu et al. 2020).

Since we did not find evidence of the interactions between soil and leaf litter quality in the short term (i.e., 12 weeks of the experiment), it is likely that the leaf litter effect on soil quality is a process that develops over longer time periods and could involve rhizodeposition. Indeed, we did not detect effects of leaf litter quality on soil organic matter, i.e. basal respiration and microbial biomass (Tab. 1). According to the stress gradient hypothesis, it could be assumed that low quality soil would benefit more from high quality litter, thereby shifting from competition for nutrients to facilitation. Next to litter material, variation in the composition and/or diversity of the microbial community may impact soil respiration by the quantity and quality of plant root-derived inputs to the soil (Hooper et al. 2000, Eisenhauer et al. 2010) and the accumulation of soil organic matter (Huang et al. 2011, Lange et al.

2015). We caution that we did not quantify litter-derived C decomposed during our experiment, which could be done using isotopic labeling to determine the rate at which litter-derived C is incorporated into the soil (e.g., Qiao et al. 2014).

Further, our results show that soil CO_2 emissions and basal respiration decreased with time (Fig. 2, Fig. 3A), likely because no additional C sources were added to the soil during the experiment. Previous studies have shown that the early stages of decomposition (ca. 0 to 40 % mass loss) are characterized by the leaching of soluble compounds and by decomposition of soluble and non-lignified cellulose and hemicellulose (Heim & Frey 2004), which are easily used by microbial communities (Schmidt et al. 2011). This finding suggests that temporal variation in soil CO_2 emissions and soil basal respiration could be associated with the phenology of leaf and root senescence of the surrounding plant community, which is an important source of C inputs to soil.

4.2   Variation in the response of soil CO_2 emissions to leaf litter quality and leaf litter qualitymicrobial biomass interactions

As hypothesized, we found that soil CO_2 emissions increased significantly with leaf litter quality (Fig. 2B). Our results coincide with previous studies demonstrating that leaf litter quality was positively related with leaf
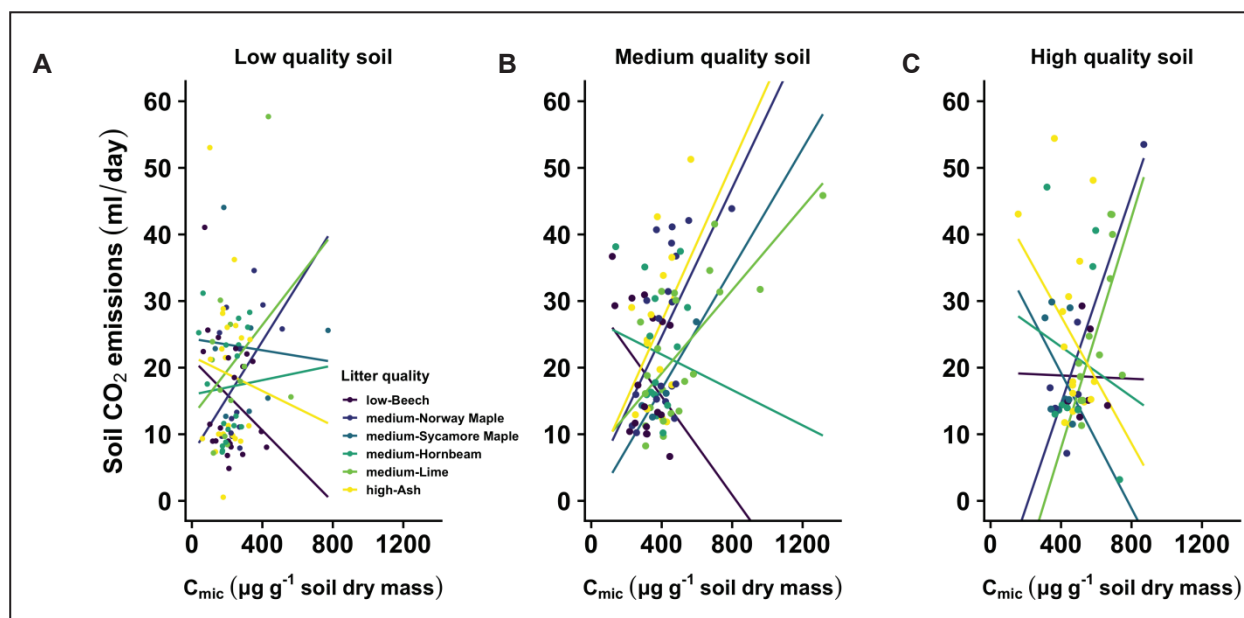

Figure 4. Relationships between soil CO_2 emissions and microbial biomass (Cmic) (**A**) in low quality soil, (**B**) in medium quality soil, and (**C**) in high quality soil across treatments with varying leaf litter quality. Lines represent each level of leaf litter quality and were fitted using linear mixed-effects models (Table 2). Points are jittered to improve visualisation. Litter quality is a continuous variable representing a gradient in litter C, N, P, Ca, and Mg concentrations, C:N, C:P, N:P, and lignin content (Fig. 1B)

litter mass loss, thus leading to losses of leaf litter C as CO_2 (Prescott et al. 1993, Cornwell et al. 2008, Vesterdal et al. 2008, Barantal et al. 2012, Mueller et al. 2015, Fanin & Bertrand 2016). Prior studies from the same site with similar leaf litter also showed that decomposition rates are positively correlated with the initial N and Ca concentrations of the leaf litter (Jacob et al. 2010a, 2010b). However, we found that leaf litter quality did not influence soil organic matter (i.e. basal respiration and microbial biomass) (Tab. 1), which may be attributable to the length of our experiment (Liu et al. 2009, Fang et al. 2015) or that other leaf litter traits are more important, e.g. specific leaf area or leaf habit (Joly et al. 2017).

Within each level of soil quality, we found a significant interactive effect of leaf litter quality and microbial biomass on soil CO_2 emissions. Contrary to our expectations, the slope of the relationship between soil respiration and microbial biomass did not strengthen with increasing leaf litter quality within each level of soil quality. In fact, we found idiosyncratic, contrasting responses of soil respiration to microbial biomass across levels of leaf litter quality. However, our measurement of soil microbial biomass does not capture potential changes in soil microbial community composition, which is likely to change with the quality of plant inputs (Lange et al. 2015, Eisenhauer et al. 2017), and likely mediates the influence of leaf litter quality on soil respiration. For example, the proportion of assimilated substrates that is used for microbial growth or soil respiration can vary in response to the fungi:bacteria (Six et al. 2006), which can be reliably predicted by leaf litter traits such as leaf litter N content (de Vries et al. 2012, Legay et al. 2014). Soil microbial community composition may reflect variation among soil microorganisms in terms of the type of leaf litter they preferentially consume (Six et al. 2006); the less protected the biomass, the more C is respired as CO_2 (Six et al. 2006). Consequently, it is likely that the variable response of soil CO_2 emissions to leaf litter quality observed in the present study did not consistently increase with increasing leaf litter quality as predicted (Fig. 4) due to the limited range of microbial biomass and changes in microbial community composition, as carbon-use efficiency often varies strongly between fungi and bacteria (Manzoni et al. 2012).

5. Conclusions

Our results suggest that changes in soil quality resulting from variation in forest diversity and/or community composition due to land-use or environment change (Augusto et al. 2002, Paterson, 2003, Russell et al. 2007,

Huang et al. 2011, Mueller et al. 2012, Xu et al. 2013) may have cascading effects on soil CO_2 emissions (Wardle 2004). Across a gradient in soil quality, leaf litter quality interacts with microbial biomass in mediating soil CO_2 emissions. Therefore, soil respiration may differ at local spatial scales due to variation in leaf litter quality and soil microbial communities. We suggest that it is important to consider local-scale biotic interactions when modeling soil carbon balance at the regional and global scales.

7. References

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Supplementary Tables 1–2

Table S1. Soil characteristics of different soil quality at 0-5 cm soil depth (means ± SE of three replicates per quality). Low soil quality is defined as soil with low C and N concentrations and low soil pH. Different letters indicate significant differences among soil quality in a given soil depth (P<0.05).

Soil Quality	C (%)	N (%)	C/N	pH (KCL)
Low	3.3 ± 0.9a	0.21 ± 0.04a	15.9 ± 1.7a	3.4 ± 0.2a
Medium	3.8 ± 1.0a	0.30 ± 0.08a	12.6 ± 0.3b	4.3 ± 0.1b
High	3.9 ± 0.9a	0.31 ± 0.08a	12.6 ± 0.7b	5.2 ± 0.1c

* Data are from Thoms et al. 2010

Table S2. Leaf litter characteristics of different leaf litter quality (mg g-1 dry mass of nitrogen (N), Carbon (C), calcium (Ca), magnesium (Mg), phosphorous (P) and lignin as well as nutrient ratios). Species are sorted according to their C:N.

	N	C	Ca*	Mg*	P*	Lignin	C/N	C/P*	N/P*
Beech	8.30	47.86	16.80	1.21	0.36	88.70	56.06	1303	23.25
Sycamore	11.02	46.71	27.49	2.86	0.61	80.70	40.08	729	18.22
Maple	11.89	48.10	27.47	2.58	0.63	75.70	37.93	720	18.96
Lime	11.78	48.21	24.62	2.10	0.63	75.80	36.53	684	18.71
Hornbeam	11.72	46.66	30.15	3.17	0.47	75.80	36.40	921	25.40
Norway Maple	15.65	49.03	28.83	2.87	0.69	80.20	27.60	631	22.86
Ash									

* Data from Jacob et al. (2009, 2010b)

References to supplementary tables 1–2

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