Soil Biology & Biochemistry 57 (2013) 390–400

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Nine years of CO_2 enrichment at the alpine treeline stimulates soil respiration but does not alter soil microbial communities

Frank Hagedorn [a,*], David Hiltbrunner [a], Kathrin Streit [b], Alf Ekblad [c], Björn Lindahl [d], Anja Miltner [e], Beat Frey [a], I. Tanya Handa [f], Stephan Hättenschwiler [f]

[a] Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), 8903 Birmensdorf, Switzerland
[b] Paul Scherrer Institute (PSI), CH-5232 Villigen PSI, Switzerland
[c] School of Science and Technology, Örebro University, SE 701 82 Örebro, Sweden
[d] Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, 75007 Uppsala, Sweden
[e] Helmholtz Centre for Environmental Research – UFZ, Department Biotechnology, 04318 Leipzig, Germany
[f] Centre of Functional Ecology and Evolution, CEFE-CNRS, 1919 route de Mende, F-34293 Montpellier, France

ARTICLE INFO

Article history:
Received 24 April 2012
Received in revised form
23 July 2012
Accepted 3 October 2012
Available online 25 October 2012

Keywords:
Carbon cycling
Elevated CO_2
Fungi
PLFA
Microbial community
Soil respiration
Isotope tracing
Treeline
Tree species

ABSTRACT

Elevated atmospheric CO_2 was often shown to stimulate belowground C allocation, but it is uncertain if this increase also alters the structure of soil microbial communities. Here, we assessed the effects of nine years of CO_2 enrichment on soil microbial communities of an alpine treeline ecosystem with 35-year-old *Larix decidua* and *Pinus mugo* ssp. *uncinata* trees. We also tracked the ^{13}C signal of supplemental CO_2 in soil-respired CO_2, microbial biomass, and phospholipid fatty acids (PLFA) in undisturbed mor-type organic layers. We found a persistently increased soil CO_2 efflux (+24% on average), but negligible effects of elevated CO_2 on the biomass and community structure of soil microorganisms under both tree species determined with PLFA and T-RFLP (terminal restriction fragment length polymorphism). The ^{13}C tracing over 9 years revealed that 24–40% of the soil microbial biomass was composed of 'new' plant-derived C. PLFA from gram-negative biomarkers did not significant shift in ^{13}C by the CO_2 addition, while those of gram-negative bacteria were significantly altered. The highest ^{13}C signals in individual PLFA was found in the fatty acid 18:2ω6,9 with 65–80% new C, indicating that new plant-derived C was primarily incorporated by soil fungi. However, CO_2 enrichment did not affect the production of mycelia biomass and the structure and composition of the fungal communities analysed by high-throughput 454-sequencing of genetic markers. Collectively, our results suggest that C flux through the plant–soil system will be accelerated but that the biomass and composition of microbial communities will be little affected by rising atmospheric CO_2 in organic matter rich treeline soils.

1. Introduction

The continued rise in atmospheric CO_2 concentration is expected to influence the structure and function of soil microbial communities, although the magnitude and even the direction of these changes remain uncertain. Effects of elevated CO_2 may arise via changes in plant biomass production and C allocation to plant roots, associated with changes to fine root turnover, root exudation and/or C export to mycorrhiza (Zak et al., 2000a; Carney et al., 2007). Many CO_2 studies reported increasing fine root biomass and soil respiration under elevated CO_2 (e.g. Comstedt et al., 2006; Jackson et al., 2009; Dieleman et al., 2012) providing evidence for higher belowground C inputs. In parallel, some experiments have shown increasing abundances of fungi and gram-negative bacteria (Janus et al., 2005; Lipson et al., 2005; Carney et al., 2007; Anderson et al., 2011), while other studies observed no changes in microbial community structure in response to elevated CO_2 (Zak et al., 2000b; Niklaus et al., 2003; Austin et al., 2009). The varying effects of increasing atmospheric CO_2 on microbial communities may be related to varying environmental conditions and constraints among different study systems, and/or to plant species-specific responses to elevated CO_2. For instance, in a review, Zak et al. (2000a) suggested that microbial biomass is not likely to change when increases in root production under elevated CO_2 are small compared to soil organic matter stocks.

By far, the largest numbers of studies observing altered microbial communities and their functions under elevated CO_2 have been conducted in mesocosms, agricultural ecosystems or in plantations

* Corresponding author. Tel.: +41 44 7392463; fax: +41 44 7392215.
E-mail addresses: hagedorn@wsl.ch, frank.hagedorn@wsl.ch (F. Hagedorn).

0038-0717/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.soilbio.2012.10.001

on formerly agricultural land (Zak et al., 2000b; Janus et al., 2005; Drissner et al., 2007; Anderson et al., 2011). However, rather small CO_2 effects on belowground activity and microbial community structure have been observed in a low fertile and mature temperate forest (Bader and Körner, 2010) and in a closed stand of a sweetgum plantation (Austin et al., 2009). This suggests that the responsiveness of soil microbial communities depends on the nutrient status and successional stage of ecosystems, likely as a result of the resource-dependent CO_2 effects on plants (Körner, 2006). Our study was conducted in an alpine treeline ecosystem, characterized by a low productivity, nitrogen-poor conditions, and soils that are weakly weathered and that have thick organic layers (Bednorz et al., 2000; Kammer et al., 2009). Little is known in general about the structure and function of microbial communities in soils at high altitudes (Hagedorn et al., 2010a). However, the common increase in the abundances of fungi with decreasing soil fertility (Högberg et al., 2007) and with increasing altitude in alpine grassland soils (Margesin et al., 2009), suggests that fungi are an important component of microbial communities at the altitudinal treeline. Because fungi appear to be particularly responsive to elevated CO_2 (Lipson et al., 2005; Billings and Ziegler, 2008; Anderson et al., 2011), CO_2 enrichment at the treeline may have strong effects on the structure and activity of these fungi-dominated microbial communities. On the other hand, the slowly growing treeline vegetation, with low C inputs into organic matter rich soils may suggest a limited potential of plant-mediated effects on soil organisms, making predictions on how soil microbial communities will respond to elevated CO_2 at the alpine treeline difficult.

The ^{13}C tracing into phospholipid fatty acids (PLFA) as specific membrane components of living cells from distinct microbial groups (Zelles, 1999; Bååth, 2003) is a promising approach to assess C dynamics within soil microbial communities in CO_2 enrichment experiments (Billings and Ziegler, 2008). The added CO_2 originating from fossil fuel combustion is depleted in ^{13}C. This depletion allows the tracking of 'new' plant-derived C into soil microbial biomass and provides the possibility to identify which microbial groups are primarily metabolizing new plant-derived C, and which groups utilize rather older SOM-derived C (Billings and Ziegler, 2008; Denef et al., 2007). Most of the ^{13}C tracer studies were conducted in microcosms or in young ecosystems (Carney et al., 2007, Denef et al., 2007), while few studies have tracked ^{13}C labelled new C into microbial communities at the ecosystem scale after long-term CO_2 enrichment (Billings and Ziegler, 2008). These few studies indicated that fungi incorporated larger fractions of new plant C than other groups of microorganisms, because symbiotic mycorrhizal fungi are directly supplied by recent photoassimilates (Butler et al., 2003; Högberg et al., 2010) and saprophytic fungi play a predominant role in litter decomposition (Esperschütz et al., 2011). The strong use of new plant C by fungal communities may also explain why they respond most sensitively to elevated atmospheric CO_2.

Here we assessed the effects of nine years of CO_2 enrichment on the abundance, community structure, and activity of soil microorganisms in an alpine treeline ecosystem with undisturbed soils in the Swiss Alps. We also traced the ^{13}C signal of added CO_2 in soil respired CO_2, microbial biomass, and individual PLFA of the organic layer, allowing to understand C cycling between plants, soils and their microbial communities in more detail. The CO_2 experiment was carried out with two different tree species *Larix decidua* and *Pinus mugo* ssp. *uncinata*, both planted 35 years ago. In this study, we aimed to understand how nine years of CO_2 enrichment affected soil microbial communities and soil respiratory activity. We expected that fungal communities would show a particularly sensitive response to elevated CO_2 as they are most closely linked to recent assimilates. We also hypothesized that tree species affect the structure and activity of soil microorganisms, but the direction was difficult to predict. On the one hand, we might expect that microbial responses follow responses of litter inputs into soils which are primarily driven by plant biomass production. While *Larix* showed a positive above-ground growth response to CO_2 enrichment, the growth of *Pinus* did not change (Handa et al., 2006; Dawes et al., 2011a). On the other hand, *Pinus* (similar as *Larix*) maintained a higher photosynthetic uptake throughout the nine treatment years (Dawes et al., in press), but did not invest the additionally assimilated C into growth. This suggests that a large fraction of this additional C was allocated to the belowground, which in turn would imply potentially greater CO_2 effects on microbial communities under *Pinus* than under *Larix*.

2. Materials and methods

2.1. Study site description

The study was carried out at 2180 m a.s.l. at Stillberg in the Central Alps near Davos, Switzerland (47°28′ N, 7°30′ E). Long-term average weather data at this site since the 1950s show a mean annual precipitation of 1150 mm, mean annual maximum snow depth of 1.46 m, and average temperatures during the growing season ranged between 7 and 10.6 (Barbeito et al., 2012). The terrain is rather steep, with slopes of 25–30° that are northeast exposed. Soils are Ranker and Podzols (Lithic Cryoumprepts and Typic Cryoorthods) with sand contents in the mineral soils above 75%. The organic layers are dominated by Oa horizons that are 3–15 cm thick across the whole study site (Bednorz et al., 2000). Soil characteristics are given in Table 1.

2.2. Experimental set-up

Long-term afforestation experiment. In 1975, 92,000 of seedlings of *Larix decidua* Mill. *Pinus cembra*, and *Pinus mugo* ssp. *uncinata* (DC) were planted on an area of 5 ha on the slope of Stillberg (Barbeito et al., 2012). The CO_2-study site is located on the upper

Table 1
Properties of the organic layer (Oa horizon, 0–5 cm depth) of the treeline ecosystem at 2200 m a.s.l., Stillberg, Switzerland. Effects of tree species and CO_2 enrichment. Means and standard error of 10 plots per tree species and CO_2 level (total $n = 40$).

		pH ($CaCl_2$)[a]	Soil organic C[a] %	C/N[a] Mass ratio	Extractable[b] NH_4^+ mg N kg^{-1}	Extractable[b] NO_3^- mg N kg^{-1}
Larix decidua	Ambient CO_2	4.19 ± 0.10	40.2 ± 1.7	24.9 ± 0.8	11.7 ± 2.0	0.15 ± 0.02
	Elevated CO_2	4.29 ± 0.09	39.2 ± 1.6	24.9 ± 0.7	13.4 ± 3.3	0.21 ± 0.04
Pinus uncinata	Ambient CO_2	4.28 ± 0.15	38.0 ± 2.0	26.2 ± 0.5	9.5 ± 1.5	0.20 ± 0.05
	Elevated CO_2	4.24 ± 0.08	40.4 ± 1.7	26.0 ± 0.4	10.0 ± 1.6	0.19 ± 0.03
P_{CO_2}		n.s.	n.s.	n.s.	n.s.	n.s.
$P_{species}$		n.s.	n.s.	n.s. (0.10)	n.s.	n.s.

[a] Means of 2003, 2005, and 2009.
[b] Means of 2004, 2005, 2007, 2009 (extracted with 0.5 M K_2SO_4).