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The impact of long-term elevated CO_2 on C and N retention in stable SOM pools

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Abstract Elevated atmospheric CO_2 frequently increases plant production and concomitant soil C inputs, which may cause additional soil C sequestration. However, whether the increase in plant production and additional soil C sequestration under elevated CO_2 can be sustained in the long-term is unclear. One approach to study C–N interactions under elevated CO_2 is provided by a theoretical framework that centers on the concept of progressive nitrogen limitation (PNL). The PNL concept hinges on the idea that N becomes less available with time under elevated CO_2. One possible mechanism underlying this reduction in N availability is that N is retained in long-lived soil organic matter (SOM), thereby limiting plant production and the potential for soil C sequestration. The long-term nature of the PNL concept necessitates the testing of mechanisms in field experiments exposed to elevated CO_2 over long periods of time. The impact of elevated CO_2 and ^{15}N fertilization on *L. perenne* and *T. repens* monocultures has been studied in the Swiss FACE experiment for ten consecutive years. We applied a biological fractionation technique using long-term incubations with repetitive leaching to determine how elevated CO_2 affects the accumulation of N and C into more stable SOM pools. Elevated CO_2 significantly stimulated retention of fertilizer-N in the stable pools of the soils covered with *L. perenne* receiving low and high N fertilization rates by 18 and 22%, respectively, and by 45% in the soils covered by *T. repens* receiving the low N fertilization rate. However, elevated CO_2 did not significantly increase stable soil C formation. The increase in N retention under elevated CO_2 provides direct evidence that elevated CO_2 increases stable N formation as proposed by the PNL concept. In the Swiss FACE experiment, however, plant production increased under elevated CO_2, indicating that the additional N supply through fertilization prohibited PNL for plant production at this site. Therefore, it remains unresolved why elevated CO_2 did not increase labile and stable C accumulation in these systems.

Keywords Elevated CO_2 · N-15 · Labile and stable SOM pools · Soil N retention · Soil C sequestration

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Abbreviations

(FACE) Free air carbon dioxide enrichment
(SOM) Soil organic matter
(PNL) Progressive nitrogen limitation

Introduction

The atmospheric CO_2 concentration has been increasing continuously since the industrial revolution, and it is expected to continue rising due to extensive burning of fossil fuels and land-use changes (Houghton and Ding 2001). Elevated atmospheric CO_2 will likely affect agro-ecosystem functioning through its direct impact on photosynthesis. Indeed, plant production has increased by 20% on average under elevated CO_2 (Ainsworth and Long 2005; de Graaff et al. 2006b). Gifford (1994) suggested that increased C assimilation by plants and its subsequent sequestration in the soil may counterbalance the rise in CO_2 emissions. However, enhanced soil C sequestration under rising levels of CO_2 can only occur if increases in soil C input are sustained (Taylor and Lloyd 1992; Friedlingstein et al. 1995; Kicklighter et al. 1999) and soil C mineralization lags behind the increase in soil C input (Raich and Schlesinger 1992).

The rates of both soil C input and mineralization are strongly controlled by soil N availability; however, the impact of elevated CO_2 on soil N mineralization processes is uncertain (Norby and Cotrufo 1998; Zak et al. 2000). Most studies have observed that elevated CO_2 initially stimulates plant production and concomitant soil C inputs, but these enhanced soil C inputs either stimulated or reduced soil N mineralization. For example, Zak et al. (1993) showed that the increased C inputs under elevated CO_2 stimulated the growth of soil microbial biomass, thereby increasing rates of N mineralization. Whereas, Diaz et al. (1993), found that increased C inputs under elevated CO_2 stimulated competition between the soil microbial biomass and plants for soil N, leading to a decline in soil N availability. In addition, Oren et al. (2001) showed that increased N fertilization may offset the decline in N availability under elevated CO_2. Thus, it remains unclear how initial increases in soil C input under elevated CO_2 affect microbial N transformation processes.

Another topic of debate is how the increase or decline in N availability ultimately controls soil C sequestration. Results of a recent meta analysis showed that if plenty of N is available, enhanced plant growth and soil C input under elevated CO_2 are likely sustained, resulting in net soil C sequestration (de Graaff et al. 2006b; Reich et al. 2006; van Groenigen et al. 2006). However, it has also been argued that ample soil N availability may simultaneously enhance soil C mineralization (Niklaus et al. 1998). In that case, the increased CO_2 respiration rates could off-set the increase in soil C accumulation.

To explain how elevated CO_2 affects soil N availability and soil C sequestration, Luo et al. (2004) proposed a conceptual framework which can be used to study C–N interactions under elevated CO_2 conditions. They projected that an increase in C influx into an ecosystem under elevated CO_2 stimulates two processes that are critical for regulating long-term ecosystem N dynamics: (1) increased demand for N to support stimulated plant growth and (2) enhanced sequestration of N into plant biomass and long-lived SOM pools. The latter process can decrease soil N availability for plant growth and serves as the core mechanism in driving progressive N limitation (i.e. PNL). If PNL occurs, enhanced plant growth and soil C sequestration can not be sustained under elevated CO_2. Nevertheless, if N sequestration is compensated for by additional N supply through N-fertilization or N deposition, it is possible that N will not limit C accumulation at all.

To test whether elevated CO_2 does in fact increase incorporation of N and C into long-lived SOM pools, data on soil C and N dynamics under elevated CO_2 from long-term field experiments are required (Luo et al. 2004). Free Air Carbon dioxide Enrichment (FACE) techniques allow for long-term CO_2 fumigation studies in a field situation (Rogers et al. 1983; Hendrey 1993). The impact of elevated CO_2 and N fertilization on *L. perenne* and *T. repens* monocultures has been studied in the Swiss FACE experiment for 10 consecutive years. Therefore, this experiment offers a good opportunity to test the validity of the PNL concept. It has already been established that elevated CO_2 has not significantly increased soil C and N sequestration during the 10 years of this experiment (van Kessel et al. 2005). Yet, trends eluding to changes in soil C and N accumulations under elevated CO_2 have been found (Six et al. 2001; van Groenigen et al. 2003), and the reason that such changes have not been detected may be a result of the difficulty to detect

statistically significant changes in total soil C and N pools, since these pools with long residence times are large but change slowly (Hungate et al. 1996, Schlessinger and Lichter 2001).

The long-term use of ^{15}N fertilizer in the Swiss FACE experiment offers a unique opportunity to assess with greater sensitivity whether elevated CO_2 stimulates N accumulations in stable soil pools. Indeed, using physical fractionation, Van Groenigen et al. (2003) did detect an increase in the amount of fertilizer-N recovered in the SOM pools associated with the mineral fraction (mSOM) for both species receiving a high N fertilization rate and exposed to elevated CO_2. This finding suggests that elevated CO_2 increased N sequestration, which supports the PNL-concept. In this study we used long-term incubations to separate the 10 year long ^{15}N-fertilizer addition into stable and labile SOM-N pools. In addition we measured soil CO_2 efflux during the incubation to be able to define C incorporated in labile and stable soil C pools. Based on the PNL concept, we hypothesized that sustained increases in plant production under elevated CO_2, will stimulate the incorporation of C and fertilizer-N into stable SOM pools.

Materials and methods

Site description and sampling procedure

The Swiss Free Air Carbon dioxide Enrichment (FACE) field site at Eschikon, Switzerland was established in 1993, and consists of six FACE rings, laid out in a split–split–plot design. Three of the rings were exposed to ambient atmospheric CO_2 concentrations, while the other three rings were exposed to elevated atmospheric CO_2 concentrations (60 Pa $CO_2 \pm 10\%$ over 92% of the fumigated time). The subplots consisted of monocultures of *Lolium perenne* and *Trifolium repens*, that were cut 5 times a year, after which the harvested biomass was removed from the plots. Nitrogen fertilizer ($^{15}NH_4{}^{15}NO_3$) was applied 4 times a year at rates of 140 or 560 kg N ha^{-1} y^{-1}. The low and high N treatments contained 0.3841 and 0.1789 atom% ^{15}N excess in 1995 and 1.0602 and 0.2890 atom% ^{15}N excess from 1996–2000, respectively. The soil is classified as a fertile, eutric Cambisol consisting of clay-loam (Zanetti et al. 1996).

In March of 2003, 20 soil samples were taken from the ah-horizon to a depth of 10 cm, from each of the treatment plots. A soil core with a diameter of 7 cm allowed us to take four homogeneously distributed subsamples per subplot. Individual soil cores taken from a subplot were composited and sieved to 10 mm to facilitate future soil preparation. Subsequently, the sieved soil was air dried for 48 h prior to shipment. Following shipment, the soil was ground, sieved (<2 mm), and roots (>2 mm) were removed.

Incubation with repeated leaching

Subsamples of the soils (70 g) were incubated in plastic filters (Falcon Filter model 7111; Becton Dickinson Labware, Lincoln Park, NJ, USA) at 35°C (Kaye et al. 2002). A glass fiber filter (Whatman GF/A, Whatman Inc., Ann Arbor, MI, USA), and an "extra thick" glass fiber prefilter (Gelman Sciences, Ann Arbor, MI, USA) were used to replace the filter originally in the filter unit. Water-holding capacity of the soils was determined by calculating the difference in weight of soils at saturation point and oven-dry weight (100°C). Water was added to obtain 60% of water holding capacity. Subsequently, the filter units were sealed in airtight 2L jars fitted with septa. Ten ml of water was added to the bottom of the jar to prevent the soil from drying. Three jars containing the filter unit, but no soil were included for background N and ^{15}N measurements.

To determine the labile soil N pool size, the soils were leached at days: 1, 8, 25, 43, 58, 86, 112, 145, 175 and 220, with a leaching solution containing all essential nutrients except for N (Stanford and Smith 1972; Nadelhoffer 1990; Kaye et al. 2002). At each leaching, 120 ml of the N-free leaching solution was added to the top of the filter, allowed to equilibrate with the soil for 45 min., and then drawn through the filter with a weak vacuum until all the leachate was collected (Kaye et al., 2002). The leachates were collected in 120 ml specimen cups and frozen until further analyses for ammonium $\left(NH_4^+\right)$, nitrate $\left(NO_3^-\right)$ and nitrite $\left(NO_2^-\right)$. After the last leaching event, a 20 g subsample was taken from each of the soils and extracted with 100 ml K_2SO4 (0.5 M), after which the labile N not yet leached from the soil was determined.

Total soil C and N analyses

Subsamples of the soils (20 g) were dried ground in a ball mill and total C and N and their isotopic composition were determined by an automated N/C analyzer-isotope ratio mass spectrometer (ANCA-IRMS, Europa Scientific Integra, UK) at the UC-Davis Stable Isotope Facility.

Labile N analyses

Labile N was defined as the sum of the amounts of NH_4^+, NO_3^- and NO_2^- in the leaching solutions plus the residual labile N (NH_4^+, NO_3^- and NO_2^-) determined by K_2SO_4 extraction. The leaching solutions and extracts were analyzed colorimetrically for mineral N concentrations (Forster 1995).

Total fertilizer-N present as labile N was determined by analyzing the ^{15}N content of each of the solutions after each leaching event. The ^{15}N determination was performed by diffusing N from the leaching solutions and extracts onto acidified disks sealed in Teflon tape (Stark and Hart 1996). A 10 ml subsample of each of the solutions was transferred to a 20 ml plastic scintillation vial. Both MgO and Devarda's alloy were added to convert NO_3^- and NH_4^+ to NH_3, and collected on two disks (Whatman #42 filterpaper) containing 7 μl of KH_2SO_4 and sealed in Teflon tape. After 5 days of diffusion, facilitated by gentle shaking, the disks were dried in an oven, packed in tin capsules, and analysed for isotopic composition by an automated N/C analyser-isotope ratio mass spectrometer (ANCA-IRMS, Europa Scientific Integra, UK) at the UC Davis stable Isotope facility.

Labile and stable fertilizer derived N calculations

The atom% ^{15}N of samples was compared to ^{15}N standards and corrected for N in diffusion reagents using the ^{15}N pool dilution method as described by Stark and Hart (1996). The mass of fertilizer derived N residing in the labile pool was calculated using the following equations (Kaye et al. 2002):

$$N_o = N_a + N_n \quad (1.1)$$

Rearranging:

$$N_n = N_o - N_a \quad (1.2)$$

$$N_o{}^*{}^{15}N_o = N_a{}^*{}^{15}N_a + N_n{}^*{}^{15}N_n \quad (1.3)$$

Substituting from Eq. 2:

$$N_o{}^*{}^{15}N_o = N_a{}^*{}^{15}N_a + (N_o - N_a){}^*{}^{15}N_n \quad (1.4)$$

Rearranging:

$$N_a = \left(N_o{}^*{}^{15}N_o - N_o{}^*{}^{15}N_n\right) / \left({}^{15}N_a - {}^{15}N_n\right) \quad (1.5)$$

where N_o is the mass of labile N, N_a is the mass of fertilizer derived N in the labile pool, N_n is the mass of labile native soil N, $^{15}N_o$ is the atom% ^{15}N excess in the leachate sample, $^{15}N_a$ is the atom% ^{15}N excess of the added N, and $^{15}N_n$ is the atom% ^{15}N of the native soil N (0.368%).

The amount of fertilizer N in the stable pool was determined by subtracting the total amount of fertilizer derived N in the labile pool (N_a) at termination of the incubation, from the amount of fertilizer derived N in the soil prior to incubation. This gives a relative estimate of the more stable pool (Paul et al. 1999; Paul et al. 2001). The amount of fertilizer derived N in the soil prior to the incubation was calculated using similar equations:

$$N_t = N_f + N_n \quad (2.1)$$

Rearranging:

$$N_n = N_t - N_f \quad (2.2)$$

$$N_t{}^*{}^{15}N_t = N_f{}^*{}^{15}N_f + N_n{}^*{}^{15}N_n \quad (2.3)$$

Substituting from Eq. 2:

$$N_t{}^*{}^{15}N_t = N_f{}^*{}^{15}N_f + (N_t - N_f){}^*{}^{15}N_n \quad (2.4)$$

Rearranging:

$$N_f = \left(N_t{}^*{}^{15}N_t - N_t{}^*{}^{15}N_n\right) / \left({}^{15}N_f - {}^{15}N_n\right) \quad (2.5)$$

where N_t is the mass of total N, N_f is the mass of the fertilizer N in the total N pool, N_n is the mass of native N in the total N pool, $^{15}N_t$ is the atom% ^{15}N excess in the total N sample, $^{15}N_f$ is the atom% ^{15}N excess of the added N, and $^{15}N_n$ is the atom% ^{15}N of the native soil N (0.368%).

Labile and stable soil C pools

The labile C pool size was estimated by measuring soil CO_2 evolution at days: 1, 2, 3, 5, 9, 15, 26, 44, 62, 95, 121, 150, 186 and 220 in the incubation jars. A septum in the lid of the jars allowed gas samples (12 ml) to be removed with a syringe and collected in 12 ml vacutainers (Labco Unlimited, Buckinghamshire, UK). Control jars (three) without soil present were included to determine the background levels of CO_2. Following gas sampling, the caps were removed and the Mason jars were flushed in open air for 30 min. To promote flushing and air exchange, jars were placed under a fan. Labile C was defined as the sum of all CO_2–C respired during the incubation. The concentration of CO_2 was determined at the University of California – Davis Stable Isotope Facility using a continuous flow, isotope mass spectrometer (PDZ Europa TGII trace gas analyzer and Geo 20–20 isotope ratio mass spectrometer, Cheshire, UK). Carbon mineralization data are expressed on the basis of oven-dry (40°C) weight of soil. Stable C was defined as total C minus labile C.

Statistical analyses

The results were analyzed using the Mixed Model in the SAS system for Windows V8. An ANOVA was conducted with blocks (i.e. the field plots) as random effects and treatments (i.e. CO_2, N and species treatments) as fixed effects. Statistical tests were performed on cumulative respired CO_2 and total labile and stable C and N at the end of the incubation. Differences between means were tested using least significant differences. The level of significance was $P=0.05$.

Results

Total labile and stable soil N

After 220 days of incubation, the total amount of labile N leached from the soils amounted to an average of 10.9% of total soil N and was not significantly affected by CO_2-, N-, or plant species treatments (Table 1). The total amount of N residing in the stable pool after 220 days of incubation amounted up to 89.1% of total soil N and was also not affected by CO_2 concentrations, N fertilization rates or plant species (Table 1).

Labile and stable fertilizer derived N

The total amount of fertilizer derived N in the soils prior to the incubation was on average 9.7% of the total soil N content and was significantly affected by the N- and species treatments, but not by the CO_2 treatments (data not shown, but equivalent data reported in van Kessel et al. 2005). Incubating the soils for 220 days with repeated leaching revealed that, on average, labile fertilizer-N was 27.6% of the

Table 1 Total soil N contents, labile soil N contents, and stable soil N contents in *T. repens* and *L. perenne* soils following 10 years of low and high N fertilization and ambient and elevated CO_2

Species treatment	CO_2-treatment	N-treatment	Total N (mg g^{-1} soil)	Labile N (mg g^{-1} soil)	Stable N (mg g^{-1} soil)
L. perenne	Ambient	Low	3.25±0.05	0.34±0.02	2.91±0.02
		High	3.24±0.14	0.33±0.05	2.91±0.09
		Low	3.04±0.50	0.28±0.03	2.76±0.47
	Elevated	High	2.99±0.39	0.38±0.02	2.62±0.37
T. repens	Ambient	Low	2.92±0.24	0.34±0.03	2.58±0.21
		High	3.47±0.22	0.38±0.03	3.09±0.18
	Elevated	Low	3.49±0.18	0.40±0.05	3.09±0.13
		High	3.39±0.48	0.32±0.03	3.07±0.45
Source of variation ANOVA					
CO_2			ns	ns	ns
Species			ns	ns	ns
N			ns	ns	ns
CO_2 × species			ns	ns	ns
CO_2 × N			ns	ns	ns
CO_2 × Species × N			ns	ns	ns

Mean±SEM ($n=3$)

total fertilizer-N pool; the remaining 72.4% was stable fertilizer-N. Elevated CO_2 did not change the total amount of fertilizer derived N leached from the soils compared to ambient CO_2 (Fig. 1a,b; Table 2). However, significantly more fertilizer derived N was leached from the *L. perenne* than from the *T. repens* soils (Fig. 1a,b; Table 2). In addition, significantly more labile fertilizer derived N was leached from the soils receiving high N fertilization rates compared to the soils receiving low N fertilization rates (Fig. 1a,b; Table 2).

Total amounts of stable fertilizer derived N residing in the soils at day 220 were significantly affected by CO_2-, species and N-treatments. Stable fertilizer derived N contents were significantly lower for both *T. repens* and *L. perenne* soils when the soils had received low N fertilization rates (by 34.8% on average) (Fig. 1c,d; Table 2). Elevated CO_2 significantly stimulated stable fertilizer N formation in the *L. perenne* soils receiving low and high N fertilization rates and *T. repens* soils receiving low N fertilization treatments (Fig. 1c,d; Table 2).

Microbial activity

Elevated CO_2 did not alter cumulative microbial CO_2 respiration in the *L. perenne* soils receiving the low and high N fertilization rates (Fig. 2a; Table 2). Cumulative CO_2 respiration from *T. repens* soils was equal for the soils receiving high N fertilization rates in both ambient and elevated CO_2 treatments (Fig. 2b; Table 2). Also, the CO_2 respiration from the *T. repens* soils receiving the low N fertilization rates was similar under both ambient and elevated CO_2 (Fig. 2b; Table 2). Cumulative CO_2 respiration from the *T. repens* soils receiving the low N fertilization rates under elevated CO_2 was significantly smaller than the respiration from the soils receiving the high N fertilization rates under both ambient and elevated CO_2 (Fig. 2b; Table 2). Cumulative CO_2 respiration from the *T. repens* soils previously exposed to elevated CO_2 and receiving low N fertilization rates was significantly lower than cumulative CO_2 respiration from the *L. perenne* soils exposed to elevated CO_2 and receiving high N fertilization rates (Fig. 2a,b; Table 2).

The amount of labile C respired from the soil during 220 days of incubation amounted to 8.1% of total soil C on average, and was not affected by any of the treatments (Table 3). The amount of more stable soil C was on average 91.9% of total soil C, and was also not affected by exposure to ambient or elevated CO_2, low or high N fertilization rates or plant species (Table 3).

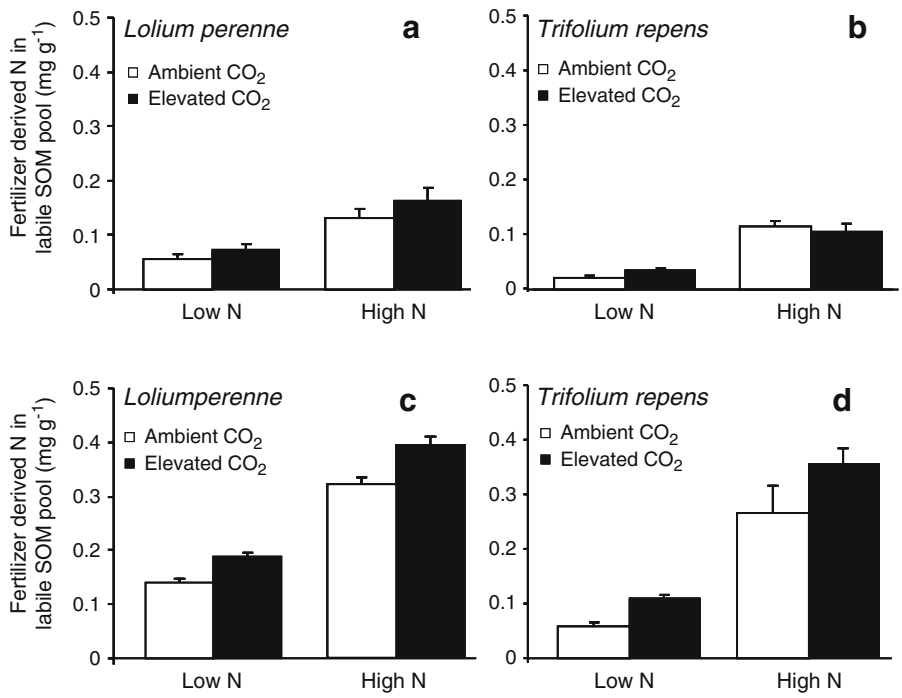

Fig. 1 **a** Labile fertilizer derived N in *L. perenne* soils following 10 years of ambient versus elevated CO_2 and low versus high ^{15}N–N fertilization rates. **b** Labile fertilizer derived N in *T. repens* soils following 10 years of ambient versus elevated CO_2 and low versus high ^{15}N-fertilization rates. **c** Stable fertilizer derived N in *L. perenne* soils following 10 years of ambient versus elevated CO_2 and low versus high ^{15}N-fertilization rates. **d** Stable fertilizer derived N in *T. repens* soils following 10 years of ambient versus elevated CO_2 and low versus high ^{15}N-fertilization rates. Values are means with SEM (n=3) indicated by the error bars

Table 2 Analysis of variation associated with the means and SEM-values represented in Figs. 1a–d and 2a–b

Source of variation ANOVA			
	Labile fertilizer derived N	Stable fertilizer derived N	CO_2 respiration
CO_2	ns	*	ns
Species	ns	*	*
N	ns	*	*
L. perenne			
CO_2	ns	*	ns
N	ns	*	ns
$CO_2 \times N$	ns	*	ns
T. repens			
CO_2	ns	*	ns
N	ns	*	ns
$CO_2 \times N$	ns	*	*

Discussion

N dynamics under elevated CO_2

We found that elevated CO_2 significantly stimulated retention of fertilizer-N in the stable SOM pools of both the *L. perenne* and *T. repens* soils. The percentage of fertilizer derived stable N residing in the soil at the end of the incubation, relative to the total amount of fertilizer derived N at inception of the incubation was, on average, 70% for the soils exposed to ambient- and 75% for the soils exposed to elevated CO_2. This suggests that a major fraction of the fertilizer derived N had been stabilized in the soils exposed to both ambient and elevated CO_2. Earlier results on N stabilization in SOM have also suggested that substantial quantities of N are incorporated into stable organic pools. For example, after a long-term incubation with repeated leaching, Kaye et al. (2002) found that 2 years after a ^{15}N tracer was applied, half of the retained N resided in the stable pool of a grassland soil.

Our data corroborate the results of van Groenigen et al. (2003), who investigated the impact of elevated CO_2 on N dynamics in the Swiss FACE swards using physical fractionation techniques. They found that elevated CO_2 increased the amount of fertilizer-N recovered in the SOM pools associated with the mineral fraction (mSOM) for both species in the high N treatments. Since, the mSOM-pool is considered the more stable SOM pool, an increase of N in these pools also suggests a potential for increased N retention under elevated CO_2. The physical fractionation technique used by van Groenigen et al. (2003) did however not estimate soil N stability directly, instead, our laboratory incubations allowed for directly determining whether the added fertilizer-N is isolated from the plant-microbe internal cycle, since N availability to microbes is directly estimated (Robertson and Paul 1999). Our results and those of van Groenigen et al. (2003) however, do compliment each other and both support the hypothesis that elevated CO_2 increases N retention into long-lived SOM pools.

Leaching of fertilizer derived N was significantly higher in the soils receiving high- compared to low N fertilization rates. Although this finding is not surprising, leaching of the labile fertilizer derived N was significantly higher in the *L. perenne*, compared to the *T. repens* soils. This suggests that the amounts of labile fertilizer N susceptible to mineralization

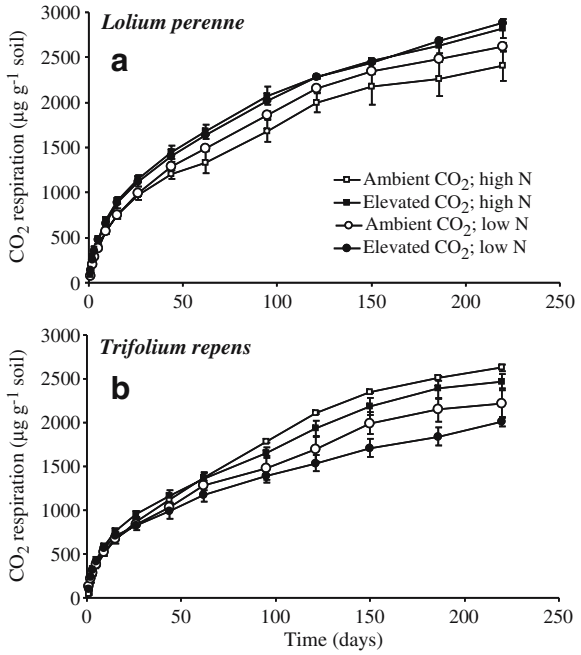

Fig. 2 **a** Cumulative CO_2 respiration during 220 days incubation from *L. perenne* soils following ambient versus elevated CO_2 and low versus high N fertilization treatments. **b** Cumulative CO_2 respiration during 220 days incubation from *T. repens* soils following ambient versus elevated CO_2 and low versus high N fertilization treatments. Values are means with SEM (n=3) indicated by the error bars

Table 3 Total soil C contents, labile soil C contents, and stable soil C contents in *T. repens* and *L. perenne* soils following 10 years of low and high N fertilization and ambient and elevated CO_2

Species treatment	CO_2-treatment	N-treatment	total N (mg g^{-1} soil)	labile N (mg g^{-1} soil)	stable N (mg g^{-1} soil)
L. perenne	Ambient	High	29.92±1.09	2.44±0.19	27.48±0.93
		Low	28.74±0.37	2.40±0.17	26.33±0.47
	Elevated	High	30.79±3.62	2.48±0.39	28.30±3.28
		Low	38.78±1.33	2.82±0.11	35.95±1.25
T. repens	Ambient	High	26.74±0.90	2.22±0.16	24.53±0.89
		Low	29.08±1.01	2.32±0.30	26.76±0.72
	Elevated	High	30.82±2.87	1.90±0.12	28.92±2.75
		Low	26.92±4.82	2.52±0.08	24.40±4.78
Source of variation ANOVA					
CO_2			ns	ns	ns
Species			ns	ns	ns
N			ns	ns	ns
CO_2 × species			ns	ns	ns
CO_2 × N			ns	ns	ns
CO_2 × species × N			ns	ns	ns

Mean±SE (n=3), Significant at the 0.05 level of probability

were larger in the *L. perenne* compared to the *T. repens*. It appears that this phenomenon was caused by differences in initial fertilizer-N contents between the soils. Namely, the loss of the fertilizer-N originally applied to the swards was greater for the *T. repens* than for the *L. perenne* systems; a total loss of 43.4% of applied ^{15}N fertilizer for *L. perenne* versus 65.8% for *T. repens* systems (van Kessel et al. 2005). Consequently, more fertilizer N in the *L. perenne* systems was available for mineralization during our incubation experiment.

Elevated CO_2, however, did not reduce leaching of fertilizer derived N in our incubation. This corroborates the results from earlier studies on N mineralization in the Swiss FACE experiment; both gross N mineralization rates measured by using the ^{15}N dilution technique in intact soil cores and mineralization rates of soil (Richter et al. 2003) and plant material during a 90 day laboratory incubation experiment were not affected by elevated CO_2 (de Graaff et al. 2006a). Yet, the lack of a difference in leaching of fertilizer derived N between soils exposed to ambient and elevated CO_2 was unexpected, since at termination of the incubation the amount of stable fertilizer derived N residing in the soils exposed to elevated CO_2 was greater than the amounts in the soils exposed to ambient CO_2. The explanation for the discrepancy between the lack of differences in loss of labile fertilizer-N and the simultaneous increase in stable fertilizer-N may be found in the insignificant differences in initial fertilizer-N contents in the soils. Indeed, the initial fertilizer-N contents in the soils showed a trend towards more fertilizer derived N retained in the soils exposed to elevated CO_2. Even though the amounts of fertilizer derived labile N were not significantly different between ambient and elevated CO_2 treatments, the differences were sufficient to cause a shift from insignificant differences between ambient and elevated CO_2 in the amounts of stable fertilizer derived N retained in the soil at inception of the incubation, to significant differences in stable fertilizer-N at termination of the incubation. The greater initial fertilizer derived N recovery in response to elevated CO_2, although not significant, did suggest a stimulating effect of CO_2 on N retention. Our data now provide direct evidence that elevated CO_2 stimulates N stabilization, which supports the PNL concept.

Linking soil N dynamics to soil C dynamics under elevated CO_2

A partial indicator of PNL is N mineralization; a decrease in N mineralization is likely to indicate incipient PNL (Luo et al. 2004). None of the studies conducted at the Swiss FACE site have detected either a significant increase or decrease in N mineralization

rates (de Graaff et al. 2006a, Richter et al. 2003). However, in this present study we found significantly more fertilizer derived N accumulated in the stable SOM pool under elevated compared to ambient CO_2. Therefore, one aspect of the PNL concept (i.e. reduced soil N availability with time) is supported. In addition, elevated CO_2 did not affect soil C sequestration; neither labile- of nor stable C were significantly affected by CO_2 concentration, N-fertilizer rates, or species. These results suggest that PNL could have prevented soil C sequestration under elevated CO_2 in the Swiss FACE experiment.

However, only the *L. perenne* system receiving low N fertilization rates and elevated CO_2 appeared limited by N availability (Daepp et al. 2000). In the *L. perenne* systems receiving the high N fertilizer treatment, biomass production had increased by 28%. In addition, biomass production of *T. repens* was enhanced under elevated CO_2 by 11–14% in both the low and high N fertilizer treatments (Aeschlimann et al. 2005). This suggests that the stimulated N sequestration under elevated CO_2 was sufficiently compensated for by the additional N supply through N fertilization and symbiotic N_2 fixation to sustain enhanced plant production. Thus, additional N inputs had prevented PNL to be expressed in plant productivity at the Swiss FACE site. Hence, PNL cannot explain the lack of soil C sequestration under elevated CO_2 in the Swiss FACE experiment.

Instead, the lack of soil C sequestration may have been caused by limited incorporation of C in long-lived SOM pools. When Six et al. (2001) used physical fractionation to detect changes in soil C sequestration under elevated CO_2, they observed a significant increase in macroaggregation. Macroaggregates, have relatively high turnover rates, therefore increased incorporation of C in macroaggregates does not necessarily enhance long-term C stabilization (Six et al. 2001). Concurrently, the fraction of new C increased only in the first three years of the Swiss FACE experiment and remained relatively stable in the following years (de Graaff et al. 2004, van Kessel et al. 2005). This suggests that during the FACE experiment, the majority of the new C was incorporated in soil pools with relatively fast turnover times, which may have prohibited net soil C sequestration under elevated CO_2.

Finally, net soil C sequestration under elevated CO_2 may have been partially prevented by the frequent removal of the harvested biomass. Root biomass production, however, was significantly promoted by elevated CO_2 (Hebeisen et al. 1997). Roots are in fact a more important contributor to soil C input than shoots (Rasse et al. 2005), and cutting increases root derived soil C inputs through enhanced root exudation (Hamilton and Frank 2001). However, the ratio of root- over shoot-derived C decreases significantly with time (Six et al. 2001), and increased labile C inputs through root exudation may stimulate microbial mineralization of SOM (Kuzyakov et al. 2000). Thus, increased root derived soil C inputs, without enhanced shoot derived soil C inputs do not necessarily elicit enhanced soil C sequestration under elevated CO_2 in the long-term.

Apparently, prevention of nutrient limitation to plant growth in fertilized agro ecosystems exposed to elevated CO_2 does not necessarily lead to enhanced soil C sequestration, since management practices may reduce soil C input or increase decomposition rates under elevated CO_2, thereby counterbalancing the stimulating effect of fertilizer applications on plant growth and concomitant soil C sequestration. Moreover, limited incorporation of plant derived C into long-lived aggregates may prevent increased soil C sequestration under elevated CO_2. To enhance our understanding of soil C dynamics under elevated CO_2 future studies should particularly focus on the impact of root derived soil C inputs on aggregation and microbial activity.

Conclusions

Elevated CO_2 increased fertilizer derived N retention in stable SOM pools, which supports the PNL concept. In addition, C accumulation in stable SOM pools was not stimulated by elevated CO_2. The increase of N retention in stable SOM pools in conjunction with the simultaneous lack of additional stable C formation under elevated CO_2, suggest that elevated CO_2 may have induced PNL in the Swiss FACE soil. However, elevated CO_2 still stimulated plant productivity, indicating that the increased formation of stable fertilizer derived N formation under elevated CO_2 was sufficiently compensated for by the additional N supply through N fertilization and symbiotic N_2 fixation. According to the PNL concept, net soil C sequestration is expected under elevated CO_2 if N availability does not limit plant growth in

the long-term. Therefore, it remains unresolved why elevated CO_2 did not lead to an increase in stable C accumulation in these pasture systems.

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