Global Change Biology (2006) **12,** 1521–1531, doi: 10.1111/j.1365-2486.2006.01186.x

Sequestration and turnover of bacterial- and fungal-derived carbon in a temperate grassland soil under long-term elevated atmospheric pCO_2

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Abstract

Temperate grasslands contribute about 20% to the global C budget. Elevation of atmospheric CO_2 concentration (pCO_2) could lead to additional C sequestration into these ecosystems. Microbial-derived C in the soil comprising about 1–5% of total soil organic carbon may be an important 'pool' for long-term storage of C under future increased atmospheric CO_2 concentrations. In our study, the impact of elevated pCO_2 on bacterial- and fungal-derived C in the soil of *Lolium perenne* pastures was investigated under free air carbon dioxide enrichment (FACE) conditions. For 7 years, *L. perenne* swards were exposed to ambient and elevated pCO_2 (36 and 60 Pa pCO_2, respectively). The additional CO_2 in the FACE plots was depleted in ^{13}C compared with ambient plots, so that 'new' (<7 years) C inputs in the form of microbial-derived residues could be determined by means of stable C isotope analysis. Amino sugars in soil are reliable organic biomarkers for indicating the presence of microbial-derived residues, with particular amino sugars indicative of either bacterial or fungal origin. It is assumed that amino sugars are stabilized to a significant extent in soil, and so may play an important role in long-term C storage. In our study, we were also able to discriminate between 'old' (> 7 years) and 'new' microbial-derived C using compound-specific $\delta^{13}C$ analysis of individual amino sugars. This new tool was very useful in investigating the potential for C storage in microbial-derived residues and the turnover of this C in soil under increased atmospheric pCO_2.

The ^{13}C signature of individual amino sugars varied between −17.4‰ and −39.6‰, and was up to 11.5% depleted in ^{13}C in the FACE plots when compared with the bulk $\delta^{13}C$ value of the native C3 *L. perenne* soil. New amino sugars in the bulk soil contributed up to 16% to the overall amino sugar pool after the first year and between 62% and 125% after 7 years of exposure to elevated pCO_2. Amounts of new glucosamine increased by the greatest amount (16–125%) during the experiment, followed by mannosamine (−9% to 107%), muramic acid (−11% to 97%), and galactosamine (15–62%). Proportions of new amino sugars in particle size fractions varied between 38% for muramic acid in the clay fraction and 100% for glucosamine and galactosamine in the coarse sand fraction.

Summarizing, during the 7-year period, amino sugars constituted only between 0.9% and 1.6% of the total SOC content. Therefore, their absolute significance for long-term C sequestration is limited. Additionally new amino sugars were only sequestered in the silt fraction upon elevated pCO_2 exposure while amino sugar concentrations in the clay fraction decreased. Overall, amino sugar concentrations in bulk soil did not change significantly upon exposure to elevated pCO_2. The calculated mean residence time of amino sugars was surprisingly low varying between 6 and 90 years in the bulk soil, and between 3 and 30 years in the particle size fractions, representing soil organic matter pools with different but relatively low turnover times. Therefore, compound-specific $\delta^{13}C$ analysis of individual amino sugars clearly revealed a high amino sugar turnover

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despite more or less constant amino sugar concentrations over a 7 years period of exposure to elevated pCO_2.

Keywords: amino sugars, compound-specific stable carbon isotope analysis, elevated atmospheric CO_2 concentration, free air carbondioxide enrichment, microbial carbon sequestration and turnover

Received 15 March 2005; revised version received 15 April 2006; accepted 10 May 2005

Introduction

Increasing atmospheric CO_2 concentration (pCO_2) is of great concern due to its role in global climate warming. There is considerable atmospheric C absorption into oceans and uptake by terrestrial sinks (Schimel *et al*., 2001), however, this is insufficient to compensate for the anthropogenic C emissions into the atmosphere, primarily as CO_2, and predominantly due to fossil fuel burning, agricultural intensification, and forest conversion to farmland resulting in soil organic carbon (SOC) losses (Lal, 2004). Much research was conducted on the potential for C sequestration in soils to help in offsetting increasing concentrations of atmospheric pCO_2 (e.g. see reviews by Lal (2003, 2004)).

Although the global SOC pool to 1 m depth is small when compared with that of the oceans, estimated at 1550 and 38 000 Pg C, respectively, it is assumed that the SOC pool is about three and two times the biotic and atmospheric pools, respectively (Lal, 2003, 2004). Among terrestrial ecosystems, grasslands play an important role in the global C budget, storing approximately 20% of global SOC stocks (Batjes, 1996), and covering approximately 20% of the world's land area (Parton *et al*., 1995). Within Europe, temperate grasslands account for about 20% of the land area (Soussana *et al*., 2004). Knowledge of the response of these ecosystems to increasing atmospheric pCO_2 is therefore important for understanding the impacts of future global change on C cycling.

Elevated atmospheric pCO_2 is predicted to lead to increased C input into the soil, due to an increase in above- and belowground plant biomass production, as a result of increased photosynthesis. The storage and turnover of this additional C in soils may be affected in two conflicting ways. Firstly, an increased input of labile, available C will stimulate microbial processes leading to increased mineralization of soil organic matter (SOM). Secondly, the extra-fixed C may lead to increased SOM formation (Cotrufo & Gorissen, 1997).

The processes regulating C input and C output are dependent on a number of feedback mechanisms. Therefore, only long-term field experiments will provide reliable answers to the final effects of elevated pCO_2 on soil C sequestration. The long-term dynamics of SOM will determine the ability of soils to act as a net source or sink for atmospheric CO_2. How much C can be stored and for how long depends upon the labilty or recalcitrance of the soil C pools and their recycling (Six *et al*., 2001), the form of C stabilization (chemical/physical) and the physical location (inter/intra-aggregate vs. free) of C in the soil (Balesdent *et al*., 2000). Long-term C sequestration will only occur if soil C mineralization lags behind the increase in soil C input, and if the C input to the soil continually increases along with a sustained increase in photosynthesis. There is still no compelling evidence for a long-term sink of increased atmospheric pCO_2 in soils (Canadell *et al*., 1996). For instance, van Groenigen *et al*. (2002) reported no increase in organic C into soil aggregate fractions at two different rates of N fertilization under elevated pCO_2.

While in most cases, no significant net C sequestration into soil was found upon elevated pCO_2, the response of soil microbial population and activity is more controversially discussed in the literature. Although the soil microbial respiration generally increased under elevated pCO_2 (Hungate *et al*., 2000; Zak *et al*., 2000), the range of response of microbial biomass encompasses both large decreases (280%) and increases (100%) (Zak *et al*., 2000) and also no response (Schortemeyer *et al*., 1996; Niklaus *et al*., 2001a; de Graaff *et al*., 2004). Hungate *et al*. (2000) measured increased biomass of soil fungi in a serpentine and a sandstone annual grassland soil after 4 years of exposure to elevated atmospheric pCO_2. However, there was no effect on the bacterial biomass. Montealegre *et al*. (2002) reported increased populations of active bacteria in bulk soil of white clover (*Trifolium repens*) under elevated pCO_2, while higher atmospheric pCO_2 did not affect total and metabolic active bacteria in bulk soil of perennial ryegrass (*Lolium perenne*). Marilley *et al*. (1999) found an 85% and 170% increase in total and respiring rhizosphere bacteria, respectively.

Amino sugar concentrations are routinely applied to assess microbial contributions to SOM (Parsons, 1981; Koegel & Bochter, 1985; Joergensen *et al*., 1995; Chantigny *et al*., 1997; Ogram & Feng, 1997; Zhang *et al*., 1998, 1999; Amelung *et al*., 1999, 2001a, b, 2002; Appel *et al*., 1999; Guggenberger *et al*., 1999; Johnsson *et al*., 1999; Niemann & Scheffer, 1999; Glaser *et al*., 2000, 2004; Kaiser & Zech 2000; Kandeler *et al*., 2000; Rodionov *et al*.,

2001; Solomon *et al*., 2001; Suzuki *et al*., 2001; Dai *et al*., 2002; Turrión *et al*., 2002; Millar *et al*., 2004). The ratios of individual amino sugars and muramic acid have been used to characterize the relative contribution of the microbial community to SOM. The ratios of glucosamine to muramic acid, mannosamine to muramic acid, and galactosamine to muramic acid reflect the contribution of fungi, bacteria, and actinomycetes, respectively, to SOM (Glaser *et al*., 2004). Alternatively, glucosamine to galactosamine and glucosamine to muramic acid ratios were often used for illustrating the origin of amino sugars from different microbes (Guggenberger *et al*., 1999; Amelung, 2001). Recent investigations showed that amino sugars might be significantly stabilized in soil, representing both living and dead microbial biomass (Joergensen *et al*., 1995; Chantigny *et al*., 1997; Guggenberger *et al*., 1999; Amelung, 2001; Glaser *et al*., 2004). Therefore, the turnover time of these cell-wall constituents in soil is suggested to be much longer than that of the living microorganisms, however, until now, very little is known about the duration and degree of this stabilization and turnover.

Additionally, only a small fraction of the microbial community is active, while the majority is dormant. When microbial biomass is measured, this high 'background noise' may prevent detection of any actual microbial response to external factors. Thus, microbial responses to elevated atmospheric pCO_2 can be hard to measure. The use of stable carbon isotope (^{13}C) as a tracer is a good tool to distinguish between C already present in soil and incoming plant-derived organic C, and can be used to measure soil microorganisms actively involved in the transformation of either C source. A recently developed method using compound-specific isotope analysis ($\delta^{13}C$) of amino sugars extracted from soil was shown to be able to differentiate between old and new bacterial and fungal residues (Glaser & Gross, 2005). This methodology was applied for the first time in this study to determine the fraction of microbial C in the soil derived from atmospheric CO_2-C ('new' C, <7 years). The magnitude and duration of this C storage was also determined to investigate the potential of microbially derived residues to act as C storage compounds in the soil under future conditions of elevated pCO_2.

Materials and methods

Site description

The free air carbon dioxide enrichment (FACE) experiment was located at the Swiss Federal Institute of Technology (ETH) field station in Eschikon, 20 km NE of Zurich. The experiment studied the impact of elevated pCO_2 on a grassland meadow ecosystem. The site consisted of six 18 m diameter rings, with three control (nonfumigated) rings exposed to ambient air (36 Pa pCO_2) and three rings (fumigated) that received additional CO_2 (60 Pa pCO_2 in total). There was a 1 min average of 60 Pa $\pm$ 10% within 92% of the fumigated time ($\pm$ 20% within 99% of the time) during daylight (12 h day^{-1}) in the growing season from March to November. Fumigation began in spring when mean air temperature reached 5 °C and stopped in autumn when temperatures were below that threshold. The supplemental CO_2 was depleted in ^{13}C (approximately -48% in 1993–1994 and -45% in subsequent years) and upon mixing with atmospheric CO_2 ($\delta^{13}C = -8\%$), the CO_2 in the fumigated rings was depleted in ^{13}C by -22.8% on average when compared with ambient CO_2.

The soil was a clay loam, fertile Eutric Cambisol. The selected plots (2.8×1.9 m) contained a monoculture of *L. perenne* cv. Bastion (perennial ryegrass) established within each ring in August 1992. These plots received a low level of NH_4NO_3 fertilizer (100 kg N ha^{-1} yr^{-1} in 1993 and 140 kg N ha^{-1} yr^{-1} in subsequent years), which was applied to coincide with the cutting regime (four times in 1993–1995 and five times in 1996–2000). Further details of the experimental design are reported in Zanetti *et al*. (1997) and Hebeisen *et al*. (1997).

Soil sampling and preparation

Soil cores (0–10 cm) from *L. perenne* plots (low N fertilization, ambient and elevated atmospheric pCO_2), taken in 1993, immediately before CO_2 fumigation, and in the autumn of 1994, 1995, 1997 and 2000, after 1, 2, 4 and 7 years of fumigation, respectively, were frozen (−20 °C), thawed, air-dried at 25 °C and sieved to <2 mm. Samples were fractionated into four particle size classes (<2, 2–20, 20–250 and 250–2000 μm, representing clay, silt, fine sand and coarse sand, respectively) using the procedure of Amelung *et al*. (1998).

Amino sugar extraction and derivatization

Amino sugars were extracted and purified from soil samples according to Zhang & Amelung (1996). Briefly, to ground soil samples containing about 0.3 mg N, 100 μg myo-inositol was added as an internal standard. The samples were hydrolyzed with 10 mL of 6 M HCl at 105 °C for 8 h. The released amino sugars were separated from impurities by neutralization with 0.4 M KOH. Before derivatization, 100 μg of *N*-methyl-glucamine was added as a recovery standard.

Aldononitrile derivatives of amino sugars were prepared according to Guerrant & Moss (1984). Samples

were dissolved in 0.3 mL derivatization reagent [32 mg hydroxylamine hydrochloride mL^{-1} and 40 mg 4-(dimethylamino) pyridine mL^{-1} in pyridine–methanol (4 : 1 v/v)] and heated at 75–80 °C for 30 min. After acetylation with 1 mL acetic anhydride at 75–80 °C for 20 min, dichloromethane was added, and excess derivatization reagents were removed with four washing steps of 1 M HCl (1 mL) and distilled water (3 × 1 mL). The final organic phase was dried with dry air at ambient temperature and finally dissolved in 0.3 mL ethyl acetate-hexane (1 : 1 v/v).

Instrumentation

Compound-specific $\delta^{13}C$ analysis of individual amino sugars was performed on a Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry-Ion trap MS (GC-C-IRMS-MS) system which comprised of a Trace GC 2000 gas chromatograph (Thermo Finnigan MAT, Bremen, Germany) equipped with a split-splitless (S/SL) injector. Glass goose-neck liners, deactivated with 5% dimethylchlorosilane in toluene for at least 1 week, were used without glass wool packing. For chromatographic separation, a BPX5 capillary column (60 m × 0.25 mm, 0.25 μm film thickness of a cross-linked copolymer of 5% diphenyl- and 95% dimethylpolysiloxane; SGE, Ringwood, Vic., Australia) was used. The connection to the Combustion Interface III (Thermo Finnigan MAT) for compound-specific $\delta^{13}C$ measurements and simultaneous compound identification measurements via an ion-trap organic mass spectrometer (Polaris GCQ, Thermo Finnigan MAT) was accomplished with a VALCO cross piece. The temperature of the combustion and the reduction ovens were 940 and 600 °C, respectively. Helium (99.996% purity; Riessner, Lichtenfels, Germany) was used as carrier gas. Isotope ratios were measured using a Delta^{plus} IRMS (Thermo Finnigan MAT). Injection was done using an autosampler (AS 2000, Thermo Finnigan MAT) with a 10 μL syringe and 70 mm needle length (IVA, Meerbusch, Germany).

The optimized carrier gas flow and oven temperature programs for successful separation of aldononitrile derivatives on the BPX5 chromatography column were as follows: 1.0 mL min^{-1}constant gas flow, 2.0 μL injection volume at 1 : 10 split ratio, 250 and 120 °C injector and oven temperature, respectively, at injection time. Oven temperature was held at 120 °C for 1 min, raised to 230 °C at 20 °C min^{-1} and held isothermal for 22 min, raised to 260 °C at 20 °C min^{-1} and held isothermal for 8 min and finally increased to 300 °C at 60 °C min^{-1}, and held for 5 min. Further details on instrumental methodology can be found in Glaser & Gross (2005).

Calculation

$\delta^{13}C$ values of individual amino sugars in the soil samples were calculated from four replicate GC-C-IRMS measurements of the derivatives and four replicate EA-IRMS measurements of the derivatization reagent according to the following equation;

$$\delta^{13}C_{\text{Amino sugar}} = \frac{(N_{\text{Der}}\delta^{13}C_{\text{Der, corr}} - F - N_{\text{Acet}}\delta^{13}C_{\text{Acet}})}{N_{\text{Amino sugar}}}, \quad (1)$$

where N is the number of C atoms in the amino sugar derivative (N_{Der}), the original amino sugar molecule ($N_{\text{Amino sugar}}$) and the acetyl group (N_{Acet}) of the acetic anhydride used for derivatization. F is a correction factor compensating for any offset between EA-IRMS and GC-C-IRMS measurements (e.g. discrimination during derivatization, amount dependence, etc.). For details, see Glaser & Gross (2005). The $\delta^{13}C$ values of individual amino sugar derivatives obtained by GC-C-IRMS analysis were corrected for reference gas drift ($\delta^{13}C_{\text{Der, corr}}$) and amount dependence (Glaser & Amelung, 2002; Schmitt *et al*., 2003), and calibrated against the two internal standards myo-inositol and *N*-methylglucamine. The total precision of the calculated $\delta^{13}C$ values of the individual amino sugars was 1.2% for mannosamine and muramic acid, 1.5% for galactosamine, and 2.2% for glucosamine (Glaser & Gross, 2005).

As it is not possible to discriminate between inherent 'old' soil C and tracer-derived C by analyzing amino sugar concentrations in the bulk soil and particle size fractions, amino sugar concentrations were treated as constant. The fraction of carbon derived from the supplemental FACE CO_2 (tracer-C (x_{tracer})) in the amino sugars could then be calculated, using the compound-specific stable isotope approach

$$x_{\text{tracer}} = \frac{(\delta_{\text{sample}} - \delta_{\text{background}})}{(\delta_{\text{tracer}} - \delta_{\text{background}})}, \quad (2)$$

where δ_{sample} and $\delta_{\text{background}}$ are $\delta^{13}C$ values for individual amino sugars in the bulk soil and fractions in FACE and ambient plots, respectively, and δ_{tracer} is the $\delta^{13}C$ value of organic matter produced at elevated pCO_2, with $\delta_{\text{background}}$ and δ_{tracer} being the end members of the mixing model. As root material was considered the main source of new plant matter entering into the soil (also root exudates and decomposing litter from aboveground), the $\delta^{13}C$ value of *L. perenne* roots was used as the δ_{tracer} value. The mean $\delta^{13}C$ value of root samples from 1993, 1994, 1995, 1997 and 2000 was calculated as –40.85%. In the calculation it is assumed that no ^{13}C discrimination during feeding/assimilation of the plant material by the bacteria/fungi occurs (Andrews *et al*., 1999), and that after death their cell

wall amino sugars $^{13}C/^{12}C$ ratio would not undergo significant alteration by substitution, etc. It is also reasonable to assume that these processes would have taken place in the ambient plots to the same degree, and as FACE and ambient plots were compared using the latter as control plots, these effects would be eliminated mathematically.

Results and discussion

$\delta^{13}C$ values of individual amino sugars

Our results show that it is possible to trace the ^{13}C isotope signature of microbial residues in soil using individual amino sugars. Although a large variation in $\delta^{13}C$ values was observed in the control plots during the 7 years of field study, the mean $\delta^{13}C$ values of individual amino sugars approximately corresponded to those measured for the elevated plots for both bulk soil and particle size fractions, before CO_2 switch on in 1993 (Fig. 1). Mean $\delta^{13}C$ values of individual amino sugars varied between –17.4% and –30.6% in the control plots and elevated plots before CO_2 switch on (Fig. 1). Mannosamine generally exhibited the highest $\delta^{13}C$ values, followed by muramic acid and glucosamine while galactosamine generally showed the most negative $\delta^{13}C$ values (Fig. 1). Compared with bulk $\delta^{13}C$ values of a C3 grassland soil of around –27% (O'Leary, 1988), galactosamine is 0–3% depleted, while glucosamine, muramic acid, and mannosamine are 0–2.7%, 0.6–7.9%, and 3.8–9.6% enriched in ^{13}C, respectively (Fig. 1). From bulk isotope measurements, a ^{13}C enrichment of 1–3%

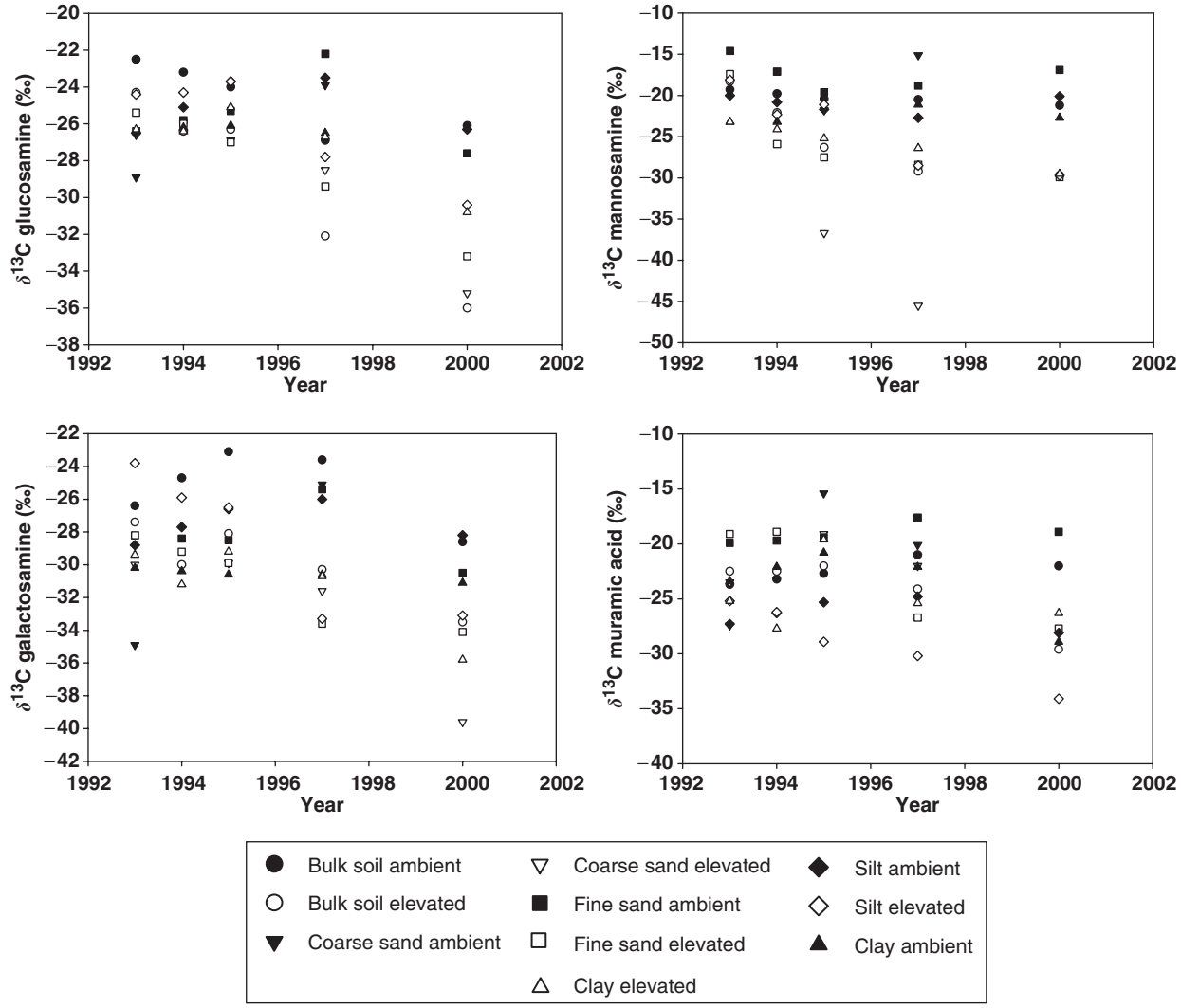

Fig. 1 $\delta^{13}C$ values of individual amino sugars in bulk soil (<2 mm, 0–10 cm) and particle size fractions during 7 years of exposure to ^{13}C-depleted elevated pCO_2 from two field replicates. Precision is given in the instrumental section.

was assumed for microbial products (Ehleringer *et al*., 2000). Compound-specific $\delta^{13}C$ measurements of individual amino sugars revealed that microbial discrimination of the heavier isotope is much more pronounced than expected. However, as mentioned in the calculation section, such discrimination will be compensated for when comparing control and FACE plots for correct quantification of microbial-derived C sequestration and turnover.

The $\delta^{13}C$ values of individual amino sugars also showed a gradual decrease over time in bulk soil and particle size fractions of the FACE plots (Fig. 1). However, for the coarse sand, a clear trend can only be depicted for glucosamine and galactosamine. This is partly due to missing values resulting from the very low material recovery of this fraction, with insufficient material for amino sugar analysis.

Amount of tracer in soil samples and particle size fractions

The differences of $\delta^{13}C$ values of individual amino sugars in the soil under ^{13}C-depleted elevated pCO_2 compared with the control plots ($\Delta^{13}C$) varied between $+0.2\%$ and -2.1% after the first year of field incubation, gradually being more negative (by 4.5–11.6%) after 7 years of exposure to ^{13}C-depleted elevated pCO_2 (Figs 1 and 2). The amount of tracer (^{13}C-depleted CO_2-C) sequestered in microbial residues and, therefore, the production of new amino sugars can be calculated as 0–16% after the first year and 62–125% after 7 years of exposure to elevated pCO_2 (Fig. 2). The percentage of tracer also indicates that relatively more glucosamine and mannosamine (about 100%) were synthesized during the experiment, followed by muramic acid (97%), and galactosamine (62%).

It is interesting to note that within different particle size fractions, there are no consistent differences between the amount of tracer and thus, the amount of microbial products synthesized during the experiment. It is generally assumed that these size fractions represent SOM pools with different turnover times (Tiessen *et al*., 1983; Bonde *et al*., 1992; Zhang *et al*., 1999). SOM separation by particle size normally shows the fastest turnover of SOM in the sand fraction and the slowest in the clay fraction (Tiessen & Stewart, 1983; Christensen, 1996). However, our results clearly indicate that following microbial breakdown of organic matter, the microbial residues remaining in the soil are not concentrated in the clay fraction, or at least not during the first 7 years.

Amounts of new amino sugars

Glucosamine was the most abundant amino sugar in bulk soil and particle size fractions followed by galactosamine, muramic acid, and mannosamine (Table 1). This is in agreement with other investigations (Amelung *et al*., 1999, 2002; Guggenberger *et al*., 1999; Solomon *et al*., 2001; Turrión *et al*., 2002; Glaser *et al*., 2004). Amino sugar concentrations in particle size fractions increased in the order coarse sand < fine sand < silt < clay (Table 1). This is commonly observed in soil and is due to the fact that SOM stability increases or SOM turnover decreases in this order (Tiessen *et al*., 1984; Guggenberger *et al*., 1994; Amelung *et al*., 2002).

It is difficult to interpret simple concentration data with respect to stability or turnover. Also in this study, during the 7 years of investigation, the concentrations of new amino sugars are highest in the clay fraction, followed by the silt and sand fractions (Table 1). Thus, in absolute terms, our results corroborate previous findings that the amount of microbes (absolute numbers or biomass) decreases with increasing particle size (Tiessen *et al*., 1984; Guggenberger *et al*., 1994; Amelung *et al*., 2002). But, the similar amount of tracer in each particle size fraction (Fig. 2) clearly reveals that microbial activity is comparable in each fraction. Cotrufo & Gorissen (1997) found a 15% increase in microbial biomass under elevated pCO_2 but only in proportion to increased root biomass.

Assuming a global temperate grassland area of 1.25×10^9 ha (Lal, 2003) and a bulk densitiy of Cambisols of about $1.36\,kg\,dm^{-3}$ (Batjes, 1996), total amounts of amino sugar C in grassland soils can be calculated as 2.7 and 2.8 Pg amino sugar-C for ambient and elevated CO_2 grassland soils, respectively (Table 1). Total SOC in temperate grassland soils was estimated as 176–295 Pg C (Batjes, 1996), thus amino sugars contribute only 0.9–1.6% to the overall SOC levels in grassland soils. This is at the lower end of a set of observations for a series of different soils carried out on individual samples on a weight basis (Glaser *et al*., 2004). Therefore, large increases in SOC under increased atmospheric pCO_2 due to microbial-derived products are unlikely. On the other hand, according to Lal (2003), the potential of world soils to sequester carbon is in the range of 0.6–1.2 Pg C. This amount is only accounted for by potential additional new amino sugar production in temperate grassland soils by 8–17%. At the same site, van Groenigen *et al*. (2003) calculated a total 'new' C input to the soil of $7803\,kg\,ha^{-1}$ for *L. perenne* with low N fertilization. From this, we can calculate that new amino sugars contributed $2625\,kg\,ha^{-1}$ or 34% to total SOC sequestration under elevated pCO_2 (Table 1). Therefore, with respect to additional C sequestration into soil under elevated pCO_2, amino sugars contribute only 8–17% but its significance with respect to elevated pCO_2-derived C sequestration into soil is much higher (34%).

Table 1 Amino sugar concentrations and stocks and new amino sugar production in bulk soil and different particle size fractions under elevated pCO_2 during a 7 years field study

			Glucosamine C			Mannosamine C			Galactosamine C			Muramic acid C			Total amino sugar C		
Sample	Year	Treatment	g kg^{-1}	kg ha^{-1}	pg in grasslands	g kg^{-1}	kg ha^{-1}	pg in grasslands	g kg^{-1}	kg ha^{-1}	pg in grasslands	g kg^{-1}	kg ha^{-1}	pg in grasslands	g kg^{-1}	kg ha^{-1}	pg in grasslands
Bulk soil	1993–2000	Mean ambient	1.88	1316	1.65	0.06	44	0.06	1.03	721	0.90	0.11	76	0.10	3.08	2158	2.7
	1993–2000	Mean elevated	1.77	1242	1.55	0.06	39	0.05	1.33	930	1.16	0.10	69	0.09	3.26	2280	2.8
	1993	New amino sugar	0.29	206	0.26	−0.01	−4	−0.01	−0.12	−87	−0.11	0.01	10	0.01	0.18	125	0.2
	1994	New amino sugar	0.52	365	0.46	0.00	−3	0.00	0.28	185	0.24	0.05	34	0.04	0.84	591	0.7
	1995	New amino sugar	0.39	272	0.34	0.00	−2	0.00	0.73	512	0.64	0.05	32	0.04	1.16	813	1.0
	1997	New amino sugar	0.86	604	0.76	0.02	11	0.01	1.08	753	0.94	0.06	43	0.05	2.02	1411	1.8
	2000	New amino sugar	1.65	1158	1.45	0.04	28	0.03	1.05	738	0.92	0.04	31	0.04	2.79	1956	2.4
Coarse sand	1993–2000	Mean ambient	0.21	149	0.19	0.01	5	0.01	0.05	38	0.05	0.01	8	0.01	0.29	200	0.3
	1993–2000	Mean elevated	0.41	286	0.36	0.00	1	0.00	0.12	87	0.11	0.02	16	0.02	0.56	390	0.5
	1993	New amino sugar	0.00	0	0.00	na	na	na	0.00	0	0.00	0.01	4	0.00	na	na	na
	1994	New amino sugar	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
	1995	New amino sugar	na	na	na	0.00	2	0.00	na	na	na	0.00	−3	0.00	na	na	na
	1997	New amino sugar	0.08	53	0.07	0.00	3	0.00	0.02	13	0.02	0.00	2	0.00	0.10	71	0.1
	2000	New amino sugar	0.33	232	0.29	na	na	na	0.11	78	0.10	na	na	na	na	na	na
Fine sand	1993–2000	Mean ambient	0.36	251	0.31	0.00	2	0.00	0.10	72	0.09	0.02	17	0.02	0.49	343	0.4
	1993–2000	Mean elevated	0.34	239	0.30	0.02	15	0.02	0.10	70	0.09	0.01	7	0.01	0.47	330	0.4
	1993	New amino sugar	−0.03	−21	−0.03	0.01	4	0.00	0.00	0	0.00	0.00	−1	0.00	−0.03	−18	0.0
	1994	New amino sugar	0.01	4	0.01	0.02	12	0.02	0.01	5	0.01	0.00	−1	0.00	0.03	21	0.0
	1995	New amino sugar	0.05	38	0.05	0.02	11	0.01	0.01	9	0.01	na	na	na	na	na	na
	1997	New amino sugar	0.23	163	0.20	0.02	13	0.02	0.08	54	0.07	0.01	6	0.01	0.34	236	0.3
	2000	New amino sugar	0.18	125	0.16	0.03	18	0.02	0.03	24	0.03	0.01	6	0.01	0.25	173	0.2
Silt	1993–2000	Mean ambient	1.33	934	1.17	0.02	13	0.02	0.63	441	0.55	0.09	62	0.08	2.07	1450	1.8
	1993–2000	Mean elevated	2.04	1427	1.78	0.04	31	0.04	1.01	710	0.89	0.09	62	0.08	3.19	2230	2.8
	1993	New amino sugar	−0.40	−282	−0.35	−0.01	−6	−0.01	−0.47	−332	−0.41	−0.02	−12	−0.02	−0.90	−631	−0.6
	1994	New amino sugar	−0.16	−109	−0.14	0.01	4	0.01	−0.17	−119	−0.15	0.00	−1	0.00	−0.32	−225	−0.3
	1995	New amino sugar	−0.01	−7	−0.01	0.00	−2	0.00	−0.01	−9	−0.01	0.03	21	0.03	0.00	3	0.0
	1997	New amino sugar	0.82	574	0.72	0.02	17	0.02	0.69	484	0.61	0.04	31	0.04	1.58	1106	1.4
	2000	New amino sugar	0.80	558	0.70	0.04	28	0.04	0.47	327	0.41	0.05	35	0.04	1.35	948	1.2
Clay	1993–2000	Mean ambient	3.56	2495	3.12	0.10	69	0.09	2.01	1405	1.76	0.31	218	0.27	5.98	4187	5.2
	1993–2000	Mean elevated	3.05	2135	2.67	0.10	71	0.09	1.64	1146	1.43	0.32	224	0.28	5.11	3576	4.5
	1993	New amino sugar	0.00	0	0.00	0.00	0	0.00	−0.13	−94	−0.12	0.05	37	0.05	−0.08	−57	−0.1
	1994	New amino sugar	0.02	12	0.02	0.01	6	0.01	0.12	82	0.10	0.13	90	0.11	0.27	191	0.2
	1995	New amino sugar	−0.35	−245	−0.31	0.03	16	0.02	−0.22	−153	−0.19	−0.12	−82	−0.10	−0.66	−463	−0.6
	1997	New amino sugar	0.11	80	0.10	0.05	35	0.04	0.03	20	0.02	0.06	42	0.05	0.25	177	0.2
	2000	New amino sugar	1.30	907	1.13	0.06	45	0.06	0.72	506	0.63	0.09	60	0.08	2.17	1518	1.9

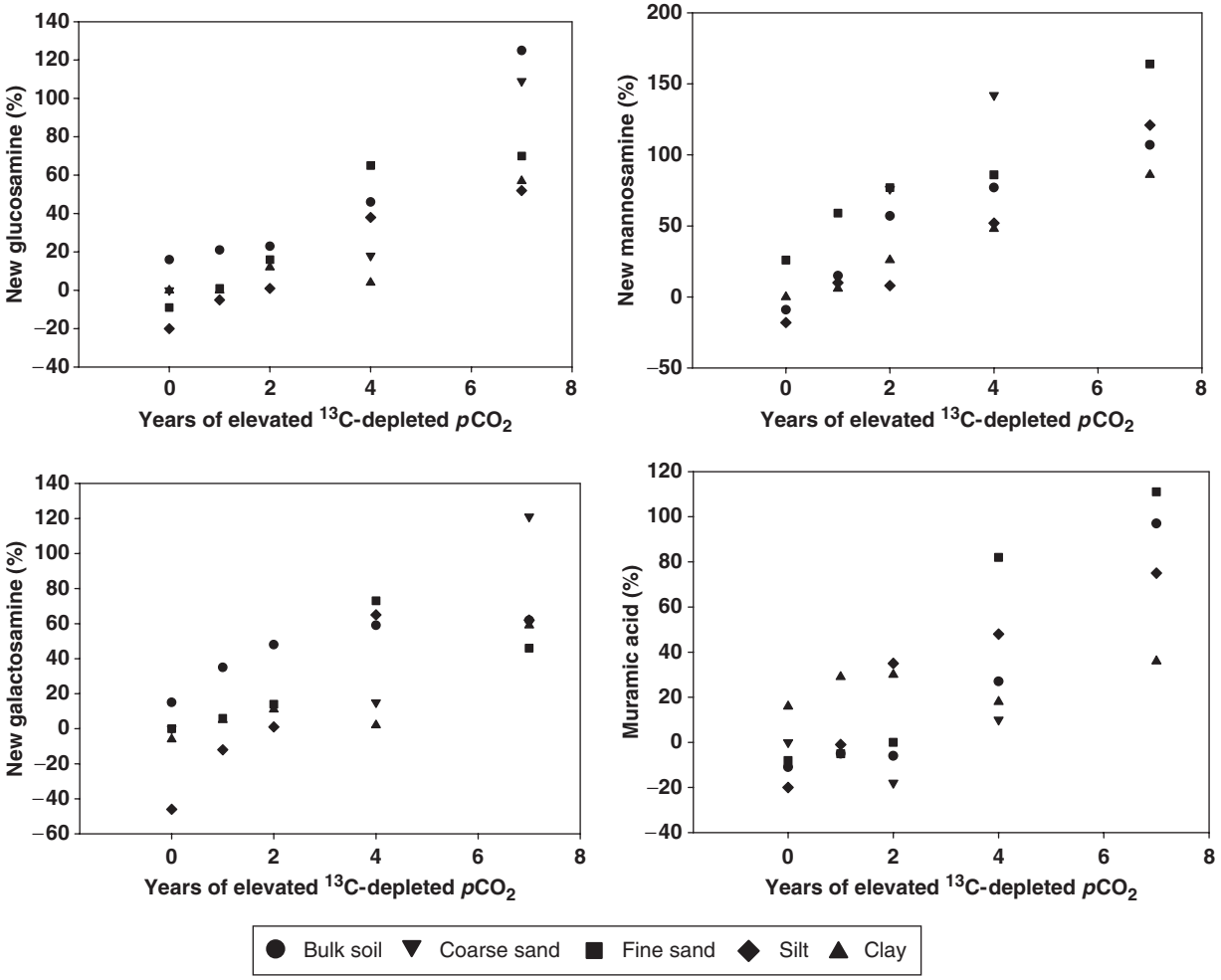

Fig. 2 Per cent tracer calculated according to Eqn (2) as $\delta^{13}C$ differences ($\Delta^{13}C$) in bulk soil and particle size fractions between plots exposed to ^{13}C-depleted elevated pCO_2 (elevated) and control plots (ambient).

Table 2 MRT of amino sugars in a temperate grassland soil and its particle size fractions under elevated pCO_2 (60 Pa)

Mean residence time (MRT) [a]	GlcN	*R*	GalN	*R*	ManN	*R*	MurAc	*R*
Bulk soil	6.2	0.95	6.1	0.99	7.9	0.96	89.9	0.97
Coarse sand	7.6	0.91	9.0	0.81	7.1	0.90	–	–
Fine sand	13.0	0.96	3.7	0.97	3.0	0.88	6.1	0.95
Silt	9.5	0.97	6.3	0.97	8.2	0.91	14.1	0.99
Clay	13.8	0.84	13.0	0.85	8.1	1.00	29.9	0.33

GlcN, glucosamine; GalN, galactosamine; ManN, mannosamine; MurAc, muramic acid.

Turnover of amino sugars under elevated pCO_2

New amino sugar formation was modeled using linear or logarithmic functions. Extrapolating these models to the actual amino sugar concentrations allowed calculating the mean residence times (MRT) of individual amino sugars in bulk soil and particle size fractions (Table 2). The MRT of amino sugars varied between 6 and 90 years in the bulk soil and particle size fractions, respectively. There was a tendency for increasing MRT with decreasing particle size (Table 2), however, the turnover of amino sugars under elevated pCO_2 is surprisingly fast. Unfortunately, there is no available literature on turnover times for microbial residues in soil,

but MRT of labile and stable SOM in temperate forest soils converted to maize cropping, was estimated at 2.6 and 45.5 years, respectively (Arrouays *et al*., 1995). The mean residence times for newly sequestered C in the soil were estimated at 1.8 and 1.1 years under *T. repens* and *L. perenne*, respectively, in this FACE experiment (van Kessel *et al*., 2000). Also, Niklaus *et al*. (2001b) reported a MRT of 1.8 years for new particulate organic matter in a calcareous grassland soil (dominated by *Bromus erectus*) in Switzerland. According to these data, amino sugars are clearly much more stable compared with particulate SOM in temperate grassland systems under elevated pCO_2. However, other studies reported MRT of 7–40 years for the light SOC fraction, representing a labile C pool, in a semiarid grassland soil (Connin *et al*., 1997). Compared with these numbers, it cannot be assumed that amino sugars belong to the stable SOM pool.

Cardon *et al*. (2001) concluded that elevated pCO_2 may increase the turnover of SOC derived from root respiration and/or from oxidation of rhizo-deposits, and reduce the microbial breakdown of old SOC. These results corroborate our relatively fast turnover of amino sugars in soil, however, in some cases, all the 'old' amino sugars were turned over after 7 years, thereby challenging the idea of the reduction of microbial breakdown of old SOC. Van Ginkel *et al*. (2000) reported that the turnover of plant-derived ^{14}C in soil was not affected by elevated pCO_2 levels. They further stated that substrate use efficiency was unaltered and soil microorganisms transform organic matter from unaltered and elevated pCO_2 in the same way.

Conclusions

Our results show that elevated pCO_2 does not significantly increase the net sequestration of microbial residues in a temperate grassland soil. Instead, the turnover of microbial residues in the soil was surprisingly fast under elevated pCO_2, with up to 100% of the microbial residues derived from elevated (^{13}C-depleted) pCO_2 turned over within 7 years. Our results unambiguously prove the assumption that microbial residues are stabilized in SOM pools in the order sand < silt < clay. This stabilization is due to the fact that increasing numbers or biomass of microbes is found in this same order, while microbial activities are similar in different particle size fractions and, thus, different SOM pools. To our knowledge this is the first time where turnover rates of specific microbial residues in soil were quantified using compound-specific stable isotope analysis without additional ^{14}C analysis. This approach is highly encouraged for further studies on SOM compounds such as bacterial- and fungal-derived C in soil under various environmental conditions and is likely to save important resources such as time and money when compared with ^{14}C analysis.

Acknowledgements

We thank Tanja Gonter and Simone Gross for help in soil sample preparation and amino sugar derivatization. Ingo Lobe is acknowledged for bringing together the principal authors. We greatly acknowledge Prof. Dr Wolfgang Zech for providing the laboratory infrastructure. Funding for this research was provided by the German Research Foundation (GL 327/4-3,4) sponsoring the stable isotope facility at Bayreuth. The Swiss FACE experiment was supported by the Swiss Federal Institute of Technology (ETH), the Swiss National Science Foundation, the Federal Offices for Agriculture, for Energy, and for Science and Education (COST) and the Brookhaven National Laboratory.

References

Amelung W (2001) Methods using amino sugars as markers for microbial residues in soil. In: *Assessment Methods for Soil Carbon* (eds Lal R, Kimble JM, Follett RF, Stewart BA), pp. 233–272. Lewis Publishers, Boca Raton.

Amelung W, Kimble JM, Samson-Liebig S *et al*. (2001a) Restoration of microbial residues in soils of the conservation reserve program. *Soil Science Society of America Journal*, **65**, 1704–1709.

Amelung W, Lobe I, Du Preez CC (2002) Fate of microbial residues in sandy soils of the south african highveld as influenced by prolonged arable cropping. *European Journal of Soil Science*, **53**, 29–35.

Amelung W, Miltner A, Zhang X *et al*. (2001b) Fate of microbial residues during litter decomposition as affected by minerals. *Soil Science*, **166**, 598–606.

Amelung W, Zech W, Zhang X *et al*. (1998) Carbon, nitrogen, and sulfur pools in particle-size fractions as influenced by climate. *Soil Science Society of America Journal*, **62**, 172–181.

Amelung W, Zhang X, Flach K-W *et al*. (1999) Amino sugars in native grassland soils along a climosequence in North America. *Soil Science Society of America Journal*, **63**, 86–92.

Andrews JA, Harrison KG, Matamala R *et al*. (1999) Separation of root respiration from total soil respiration using carbon-13 labeling during free-air carbon dioxide enrichment (FACE). *Soil Science Society of America Journal*, **63**, 1429–1435.

Appel T, Pfanschilling R, Xu FL (1999) Amino acids and amino sugars extracted by EUF from a sandy soil incubated with green manure, bacterial biomass or cellulose. *Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde*, **162**, 615–622.

Arrouays D, Balesdent J, Mariotti A *et al*. (1995) Modelling organic carbon turnover in cleared temperate forest soil converted to maize cropping by using ^{13}C natural abundance measurements. *Plant and Soil*, **173**, 191–196.

Balesdent J, Chenu C, Balabane M (2000) Relationship of soil organic matter dynamics to physical protection and tillage. *Soil and Tillage Research*, **53**, 215–230.

Batjes NH (1996) Total carbon and nitrogen in the soils of the world. *European Journal of Soil Science*, **47**, 151–163.

Bonde TA, Christensen BT, Cerri CC (1992) Dynamics of soil organic matter as reflected by natural ^{13}C abundance in

particle size fractions of forested and cultivated Oxisols. *Soil Biology and Biochemistry*, **24**, 275–277.

Canadell JG, Pitelka LF, Ingram JSI (1996) The effects of elevated CO_2 on plant-soil carbon below-ground: a summary and synthesis. *Plant and Soil*, **187**, 391–400.

Cardon ZG, Hungate BA, Cambardella CA *et al*. (2001) Contrasting effects of elevated CO_2 on old and new soil carbon pools. *Soil Biology and Biochemistry*, **33**, 365–373.

Chantigny MH, Angers DA, Prevost D *et al*. (1997) Soil aggregation and fungal and bacterial biomass under annual and perennial cropping systems. *Soil Science Society of America Journal*, **61**, 262–267.

Christensen BT (1996) Carbon in primary and secondary organomineral complexes. In: *Advances in Soil Science* (eds Carter MR, Stewart BA), pp. 97–165. CRC Lewis, Boca Raton.

Connin SL, Virginia RS, Chamberlain CP (1997) Carbon isotopes reveal soil organic matter dynamics following arid land shrub expansion. *Oecologia*, **110**, 374–386.

Cotrufo MF, Gorissen A (1997) Elevated CO_2 enhances belowground C allocation in three perennial grass species at different levels of N availability. *New Phytologist*, **137**, 421–431.

Dai XY, Ping CL, Hines ME *et al*. (2002) Amino sugars in arctic soils. *Communications in Soil Science and Plant Analysis*, **33**, 789–805.

de Graaff M-A, Six J, Harris D *et al*. (2004) Decomposition of soil and plant carbon from pasture systems after 9 years of exposure to elevated CO_2: impact on C cycling and modeling. *Global Change Biology*, **10**, 1–14.

Ehleringer JR, Buchmann N, Flanagan LB (2000) Carbon isotope ratios in belowground carbon cycle processes. *Ecological Applications*, **10**, 412–422.

Glaser B, Amelung W (2002) Determination of ^{13}C natural abundance of amino acid enantiomers in soil: methodological considerations and first results. *Rapid Communications in Mass Spectrometry*, **16**, 891–898.

Glaser B, Gross S (2005) Compound-specific $\delta^{13}C$ analysis of individual amino sugars – a tool to quantify timing and amount of soil microbial residue stabilization. *Rapid Communications in Mass Spectrometry*, **19**, 1409–1416.

Glaser B, Turrión M-B, Alef K (2004) Amino sugars and muramic acid – biomarkers for soil microbial community structure analysis. *Soil Biology and Biochemistry*, **36**, 399–407.

Glaser B, Turrión M-B, Solomon D *et al*. (2000) Soil organic matter quantity and quality in mountain soils of the alay range, kyrgyzia, affected by land use change. *Biology and Fertility of Soils*, **31**, 407–413.

Guerrant GO, Moss CW (1984) Determination of monosaccharides as aldononitrile, O-methyloxime, alditol and cyclitol acetates derivatives by gas chromatography. *Analytical Chemistry*, **56**, 633–638.

Guggenberger G, Christensen BT, Zech W (1994) Land-use effects on the composition of organic matter in particle-size separates of soil: I. Lignin and carbohydrate signature. *European Journal of Soil Science*, **45**, 449–458.

Guggenberger G, Frey SD, Six J *et al*. (1999) Bacterial and fungal cell-wall residues in conventional and no-tillage agroecosystems. *Soil Science Society of America Journal*, **63**, 1188–1198.

Hebeisen T, Luscher A, Zanetti S *et al*. (1997) Growth response of *Trifolium repens* L and *Lolium perenne* L as monocultures and bi-species mixture to free air CO_2 enrichment and management. *Global Change Biology*, **3**, 149–160.

Hungate BA, Jaeger CH, Gamara G *et al*. (2000) Soil microbiota in two annual grasslands: responses to elevated atmospheric CO_2. *Oecologia*, **124**, 589–598.

Joergensen RG, Scholle G, Wolters V (1995) Die bestimmung von muraminsäure als biomarker von bakterien. *Mitteilungen der Deutschen Bodenkundlichen Gesellschaft*, **76**, 627–630.

Johnsson L, Berggren D, Karen O (1999) Content and bioavailability of organic forms of nitrogen in the O horizon of a podzol. *European Journal of Soil Science*, **50**, 591–600.

Kaiser K, Zech W (2000) Sorption of dissolved organic nitrogen by acid subsoil horizons and individual mineral phases. *European Journal of Soil Science*, **51**, 403–411.

Kandeler E, Tscherko D, Bruce KD *et al*. (2000) Structure and function of the soil microbial community in microhabitats of a heavy metal polluted soil. *Biology and Fertility of Soils*, **32**, 390–400.

Koegel I, Bochter R (1985) Amino sugar determination in organic soils by capillary gas chromatography using a nitrogen selective detector. *Journal for Plant Nutrition and Soil Science*, **148**, 260–267.

Lal R (2003) Global potential of soil carbon sequestration to mitigate the greenhouse effect. *Critical Reviews in Plant Science*, **22**, 151–184.

Lal R (2004) Soil carbon sequestration to mitigate climate change. *Geoderma*, **123**, 1–22.

Marilley L, Hartwig UL, Aragno M (1999) Influence of an elevated atmospheric CO_2 content on soil and rhizosphere bacterial communities beneath *Lolium perenne* and *Trifolium repens* under field conditions. *Microbial Ecology*, **38**, 39–49.

Millar N, Blum H, Lobe I *et al*. (2004) The effect of long-term elevated atmospheric CO_2 on soil amino sugars in ryegrass swards. In: *Grassland Science in Europe*, Vol. 9 (ed. Lüscher A), pp. 160–162. European Grassland Federation, Zurich.

Montealegre CM, van Kessel C, Russelle MP *et al*. (2002) Changes in microbial activity and composition in a pasture ecosystem exposed to elevated atmospheric carbon dioxide. *Plant and Soil*, **243**, 197–207.

Niemann A, Scheffer B (1999) Polymeric aminoacid- and aminosugar-compounds in two fen soils from northwestern Germany. *Agribiological Research-Zeitschrift Fur Agrarbiologie Agrikulturchemie Okologie*, **52**, 127–136.

Niklaus PA, Glöckler E, Siegwolf R *et al*. (2001a) Carbon allocation in calcareous grassland under elevated CO_2: a combined ^{13}C pulse-labelling/soil physical fractionation study. *Functional Ecology*, **15**, 43–50.

Niklaus PA, Wohlfender M, Siegwolf R *et al*. (2001b) Effects of six years atmospheric CO_2 enrichment on plant, soil, and soil microibal C of a calcareous grassland. *Plant and Soil*, **233**, 189–202.

Ogram A, Feng X (1997) Methods of soil microbial community analysis. In: *Manual of Environmental Microbiology* (eds Hurst C, Knudsen GR, Mclnerney MJ, Stetzenbach LD, Walter MV), pp. 422–430. AMS Press, Washington.

O'Leary M (1988) Carbon isotopes in photosynthesis: fractionation techniques may reveal new aspects of carbon dynamics in plants. *Bioscience*, **38**, 328–335.

Parsons JW (1981) Chemsitry and distribution of amino sugars in soils and soil organisms. In: *Soil Biochemistry* (eds Paul EA, Ladd NJ), pp. 197–227. Marcel Dekker, New York.

Parton WJ, Scurlock JMO, Ojima DS *et al*. (1995) Impact of climate change on grassland production and soil carbon worldwide. *Global Change Biology*, **1**, 13–22.

Rodionov A, Amelung W, Urusevskaja I *et al*. (2001) Origin of the enriched labile fraction (ELF) in Russian Chernozems with different site history. *Geoderma*, **102**, 299–315.

Schimel DS, House JI, Hibbard KA *et al*. (2001) Recent patterns and mechanisms of carbon exchange by terrestrial ecosystems. *Nature*, **414**, 169–172.

Schmitt J, Glaser B, Zech W (2003) Amount-dependent isotopic fractionation during compound-specific isotope analysis. *Rapid Communications in Mass Spectrometry*, **17**, 970–977.

Schortemeyer M, Hartwig UA, Hendrey JR *et al*. (1996) Microbial community changes in the rhizospheres of white clover and perennial ryegrass exposed to the free air carbon dioxide enrichment (FACE). *Soil Biology and Biochemistry*, **28**, 1717–1724.

Six J, Guggenberger G, Paustian K *et al*. (2001) Sources and composition of soil organic matter fractions between and within soil aggregates. *European Journal of Soil Science*, **52**, 607–618.

Solomon D, Lehmann J, Zech W (2001) Land use effects on amino sugar signature of chromic luvisol in the semi-arid part of northern Tanzania. *Biology and Fertility of Soils*, **33**, 33–40.

Soussana J-F, Loiseau P, Vuichard N *et al*. (2004) Carbon cycling and sequestration opportunities in temperate grasslands. *Soil Use and Management*, **20**, 219–230.

Suzuki S, Shimotsu N, Honda S *et al*. (2001) Rapid analysis of amino sugars by microchip electrophoresis with laser-induced fluorescence detection. *Electrophoresis*, **22**, 4023–4031.

Tiessen H, Stewart JWB (1983) Particle-size fractions and their use in studies of soil organic matter. II. Cultivation effects on organic matter composition in size fractions. *Soil Science Society of America Journal*, **47**, 509–514.

Tiessen H, Stewart JWB, Hunt HW (1984) Concepts of soil organic matter transformations in relation to organo-mineral particle-size fractions. *Plant and Soil*, **76**, 287–295.

Tiessen H, Stewart JWB, Moir JO (1983) Changes in organic and inorganic phosphorus composition of two grassland soils and their particle size fractions during 60–90 years of cultivation. *Journal of Soil Science*, **34**, 815–823.

Turrión M-B, Glaser B, Zech W (2002) Effects of deforestation on contents and distribution of amino sugars within particle-size fractions of mountain soils. *Biology and Fertility of Soils*, **35**, 49–53.

van Ginkel JH, Gorissen A, Polci D (2000) Elevated atmospheric carbon dioxide concentration: effects of increased carbon input into a *Lolium perenne* soil on microorganisms and decomposition. *Soil Biology and Biochemistry*, **32**, 449–456.

Van Groenigen K-J, Harris D, Horwath WR *et al*. (2002) Linking sequestration of ^{13}C and ^{15}N in aggregates in a pasture soil following 8 years of elevated CO_2. *Global Change Biology*, **11**, 1094–1108.

Van Groenigen K-J, Six J, Harris D *et al*. (2003) Soil ^{13}C-^{15}N dynamics in an N_2-fixing clover system under long-term exposure to elevated atmospheric CO_2. *Global Change Biology*, **9**, 1751–1762.

van Kessel C, Nitschelm J, Horwath WR *et al*. (2000) Carbon-13 input and turn-over in a pasture soil exposed to long-term elevated atmospheric CO_2. *Global Change Biology*, **6**, 123–135.

Zak DR, Pregitzer KS, King JS *et al*. (2000) Elevated atmospheric CO_2, fine roots and the response of soil microorganisms: a review and hypothesis. *New Phytologist*, **147**, 201–222.

Zanetti S, Hartwig UA, vanKessel C *et al*. (1997) Does nitrogen nutrition restrict the CO_2 response of fertile grassland lacking legumes? *Oecologia*, **112**, 17–25.

Zhang X, Amelung W, Yuan Y *et al*. (1998) Amino sugar signature of particle-size fractions in soils of the native prairie as affected by climate. *Soil Science*, **163**, 220–229.

Zhang X, Amelung W, Yuan Y *et al*. (1999) Land-use effects on amino sugars in particle size fractions of an Argiudoll. *Applied Soil Ecology*, **11**, 271–275.

Zhang XD, Amelung W (1996) Gas chromatographic determination of muramic acid, glucosamine, mannosamine, and galactosamine in soils. *Soil Biology and Biochemistry*, **28**, 1201–1206.

