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Control of soil pH on turnover of belowground organic matter in subalpine grassland

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Abstract Grasslands store substantial amounts of carbon in the form of organic matter in soil and roots. At high latitudes and elevation, turnover of these materials is slow due to various interacting biotic and abiotic constraints. Reliable estimates on the future of belowground carbon storage in cold grassland soils thus require quantitative understanding of these factors. We studied carbon turnover of roots, labile coarse particulate organic matter (cPOM) and older non-cPOM along a natural pH gradient (3.9–5.9) in a subalpine grassland by utilizing soil fractionation and radiocarbon dating. Soil carbon stocks and root biomass, turnover, and decomposability did not scale with soil pH whereas mean residence times of both soil organic matter fractions significantly increased with declining pH. The effect was twice as strong for non-cPOM, which was also stronger enriched in ^{15}N at low pH. Considering roots as important precursors for cPOM, the weaker soil pH effect on cPOM turnover may have been driven by comparably high root pH values. At pH $<$ 5, long non-cPOM mean residence times were probably related to pH dependent changes in substrate availability. Differences in turnover along the pH gradient were not reflected in soil carbon stocks because aboveground productivity was lower under acidic conditions and, in turn, higher inputs from aboveground plant residues compensated for faster soil carbon turnover at less acidic pH. In summary, the study provides evidence for a strong and differential regulatory role of pH on the turnover of soil organic matter that needs consideration in studies aiming to quantify effects of changing environmental conditions on belowground carbon storage.

Keywords Radiocarbon · Soil fractionation · Roots · XRD · POM · Turnover · ^{15}N

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Introduction

The turnover of soil organic matter in grassland soils of cold climates is strongly limited by temperature

(Anderson 1991; Townsend et al. 1995). Climatic conditions also exert control on vegetation communities, thereby altering amount and quality of the incoming plant residues (Hobbie et al. 2000). Both factors may induce accumulation of litter-like material, such as particulate organic matter (POM) in soil (Leifeld et al. 2009), that is potentially sensitive to rapid microbial oxidation once environmental conditions change. This has prompted concern about the state and vulnerability of organic matter (OM) in cold climates such as, for example, that in European mountain soils (Sjögersten-Turner et al. 2011).

However, there are various partially interacting mechanisms involved in controlling microbial kinetics, and thus residue turnover in cold grassland soils is not influenced by temperature and litter quality alone. One often overlooked quantitative environmental factor, influencing both vegetation and soil processes, is soil pH. Low pH values are common in areas such as low-productive mountainous grasslands on silicate rocks, particularly when high rainfall and heather vegetation fosters podzolization (Bouma et al. 1969; Egli et al. 2003). Soil pH is involved in many states and processes, such as enzyme activities (Sinsabaugh et al. 2008), dissolved organic carbon (DOC) and N availability (e.g. Kalbitz et al. 2000; Pietri and Brookes 2008), and litter decomposition, as ascribed to the combinatory effect of enzyme activities, decomposer community and Al^{3+} toxicity (Walse et al. 1998). Soil acidity modulates alpine microbial (Eskelinen et al. 2009) and plant communities (Budge et al. 2011; Körner 2003; Nilsson et al. 2002), and it affected compositions of both vegetation and soil microbial biomass, even 70 years after cessation of a liming experiment in subalpine grassland (Spiegelberger et al. 2006).

The regulatory role of soil pH on decomposition rates is considered to be quite strong, with rates varying by a factor of four in the pH range from 4.0 to 6.0 (Walse et al. 1998; Leifeld et al. 2008). Much of the knowledge on the regulatory role of pH is based on in vitro activities or controlled decomposition studies. Leifeld et al. (2008) used the soil radiocarbon signature along a climate and pH gradient for the quantification of the pH effect on POM turnover after correction for temperature. Hitherto, however, there is no study that explicitly addresses the role of soil pH on the turnover time of belowground OM components, such as POM or non-POM fractions in an otherwise homogeneous field situation under long-term steady-state conditions. In addition, the role of pH on turnover of roots, the most important source for soil carbon (Rasse et al. 2005), is not yet established. Given the long turnover times of OM under cold and acid conditions of many decades, even for so-called labile fractions (Leifeld et al. 2009), the issue evades being addressed by short-term controlled experiments but can be approached by using natural pH gradients that existed for long periods of time.

Here we study the role of soil pH as a modifier for soil and root carbon turnover in a steady-state environment of a subalpine permanent grassland. We hypothesise that more acidic soils should increase the mean residence times of belowground carbon and, subsequently, also affect its distribution between labile and stable soil fractions.

Materials and methods

Sites and sampling

Alp Flix (46°30′60″N, 9°39′56″E) is located in the canton of Grisons, Switzerland, at an elevation of ca. 2,100 m a.s.l. Mean annual temperature is +2.2°C, and mean annual rainfall 1,050 mm. The site is located on a gentle slope and used as a permanent cattle pasture grazed in the summer season from June to September. Soils are well-drained Leptosols and leptic Cambisols developed on granite-diorite rock with loamy to clayey-loamy texture. The vegetation is typically an acidic Geo-Montani-Nardetum pasture, comprising patches with basophilic plant species typical for a calcareous Seslerio-Caricetum sempervirentis community. Soil pH effect on vegetation community is visible, for example, at the species level, as increasing abundance of basophilic *Plantago atrata* Hoppe, *Carex ornithopoda* Wild and *Anthyllis vulneraria* L. with pH while abundance of pointers of acidity such as *Nardus stricta* L., *Arnica montana* L. or *Gentiana acaulis* L. significantly decreases with pH (Bassin "personal communication").

The centre of the unfenced pasture extends over an area of ca. 1 ha. A soil survey in 2003 with 180 subplots across the field revealed a high variability in soil pH, ranging from 3.9 to 5.9 due to the irregular presence of limestone gravel originating from the adjacent calcareous mountain tops. For further study we selected eight of these sites that span the whole pH range but share similar soil texture.

In previous work at the same pasture, a 30 $\times$ 40 cm wide, 20 cm deep soil monolith was excavated in 2003 from each of the eight locations. Afterwards pits were filled with vegetated soil monoliths from an adjacent site. Above-ground biomass yield of extracted monoliths was measured in 2004 after one growing season for the purpose of a different study (Bassin et al. 2007). Our aboveground biomass data were taken from that study. For the present work, we made use of the corresponding archived soil samples from each of these eight locations taken in 2003 and took additional samples from the same sites in 2009. In 2003, soil samples (0–10 cm) had been taken from the four outer walls of the excavated soil monolith. These soil samples were used for measuring nutrients, texture, pH, for soil fractionation and for analyzing ^{14}C in soil fractions. In 2009, the site was re-visited to take four cores (diameter 6 cm, depth 10 cm) at distance of 0.5 m from each pit wall where monoliths have been excavated in 2003. From these cores, soil bulk density and stone content were calculated and fine soil pH, C and N concentrations were measured. In addition, pH and decomposability of biomass and ^{14}C content of roots were measured. All concentrations are based on 105°C oven-dried samples.

Soil fractionation and chemical analysis

Soils were oven-dried, sieved <2 mm and analyzed for total C, N, texture (pipette method after H_2O_2 treatment) and pH (2:1 in water). All carbon and nitrogen contents were measured after combustion with an elemental analyzer (Hekatech Euro EA 3000, Wegberg, Germany). Soils were free of carbonate. Extractable nutrients (P, K, Mg, Ca) were measured after treatment with 1:10 NH_4-acetate solution (FAL 1998).

The aim of soil fractionation was to retrieve two fractions that differ systematically in their isotopic signature to allow meaningful calculation of SOC turnover times (see below). It is based on previous studies with similar objectives where this approach proved reliable (Budge et al. 2011; Conen et al. 2008; Leifeld et al. 2009). A light POM fraction >63 µm was obtained after ultrasonic dispersion of the soil <2 mm by applying an energy of 22 J ml^{-1} suspension, followed by density separation with Na-polytungstate ($d = 1.8$ g cm^{-3}) in a centrifuge. The light material was decanted and poured into a 63 µm nylon mesh bag. After decantation the sediment in each test tube was stirred, centrifuged again and supernatants were combined and rinsed with distilled water to remove the salt. We refer to this material as coarse particulate organic matter (cPOM). The remaining material was not measured for its C and N content and isotopic signature but these parameters were calculated based on the respective values of the cPOM and the bulk soil considering mass conservation (see Eq. 1). This was done because in any fractionation some material is lost, for example in solution, which may compromise the radiocarbon-derived turnover calculation. The remaining material consists of some fine POM, mineral-associated and soluble OM. We refer to the calculated differential fraction as non-cPOM.

Material <63 µm was oxidized in a solution of 1 M NaOCl (Roth AG, Reinach), adjusted to pH 8.0, at a soil-to-solution ratio of 1:50 (w/v) and agitation for 6 h at 25°C. It corresponded to 6.5% active Cl_2 (determined by iodometry). After, the samples were centrifuged at 2,000$\times g$ for 30 min and the supernatants removed. This procedure was repeated three times. After the last treatment, the centrifugation pellets were intensively washed with deionized H_2O to remove salts, and air-dried.

The specific surface area of material <63 µm, before and after oxidation with NaOCl, was determined by N_2 adsorption using a Quantachrome Nova 2200 surface analyzer and BET isotherm.

Prior to X-ray diffraction analysis (XRD) and X-ray fluorescence spectrometry (XRF), the samples were milled. Total elemental content of Fe, Mg, Al, Si, and P in the oxidized <63 µm fraction was measured using wavelengths dispersive XRF (ARL Optim'X, Thermo Electron Corp., Switzerland).

The mineralogical composition of the NaOCl oxidized fraction <63 µm was determined using XRD on randomly oriented specimens. X-ray measurements were made using a Bragg-Brentano diffractometer (BRUKER AXS D8, CuKα with theta compensating slits and graphite monochromator) in the range of 2–80°2θ with a step width of 0.02°2θ and a counting time of 2 s. A special frontloading preparation was carried out to hold the preferred orientation as low as possible in randomly oriented specimens (Kleeberg et al. 2008). In the range 2–15θ the measurements were carried with and without ethylene glycol solvation. The intercalation of ethylene glycol causes a typical shift of the (00l) reflexes in the XRD pattern of expandable clay minerals. The qualitative phase composition was determined on the basis of the peak position and the relative intensities of the mineral phases were identified in comparison to the ICDD data base. The analysis was carried out with the software package DIFFRACplus (Bruker AXS). The quantitative mineralogical composition was estimated using the peak heights of the XRD patterns of randomly oriented specimens.

Stable N isotope ratios were measured on cPOM and bulk soils by ion ratio mass spectrometry (Thermo Finnigan Delta plus XP coupled with an elemental analyzer Flash EA 1112 Series; accuracy 0.2 permil). The δ^{15}N of non-cPOM was calculated by mass balance as

$$\delta^{15}\mathrm{N}_{\mathrm{non-cPOM}} = (\delta^{15}\mathrm{N}_{\mathrm{SOM}} - (\delta^{15}\mathrm{N}_{\mathrm{cPOM}} * \mathrm{s}_{\mathrm{cPOM}}))/(1 - \mathrm{s}_{\mathrm{cPOM}}) \tag{1}$$

where $\mathrm{s}_{\mathrm{cPOM}}$ is the mass ratio of nitrogen bound in cPOM to nitrogen bound in SOM.

Bulk soil samples, cPOM, and roots that contained 0.5–1 mg of C were combusted and graphitised for AMS measurements of radiocarbon content. These were measured at the AMS facility of Laboratory of Ion Beam Physics of the Institute for Particle Physics of at the ETH (the Swiss Federal Institute of Technology), Zurich (Synal et al. 2007). The results were expressed as percent modern carbon (pMC) calculated following the protocol of Stuiver and Polach (1977). Radiocarbon content of non-cPOM was calculated by carbon mass balance in correspondence to Eq. 1.

14C-derived mean residence times of soil fractions, bulk soil, and roots

A radiocarbon bomb model based on Harkness et al. (1986), but adjusted for time-lag (TL) effects, was applied for calculating carbon mean residence times (MRTs) separately for roots, cPOM, and non-cPOM. In the model, the ^{14}C activity of the carbon can be expressed as

$$A_t = A_{(t-1)}e^{-k} + (1 - e^{-k})A_{(i-TL)} - A_{(t-1)}\lambda \tag{2}$$

where $A_{(t)}$ is the (measured) ^{14}C activity (pMC) of C in any fraction at time t, $A_{(t-1)}$ the ^{14}C activity of the previous year; $A(_i)$ is the atmospheric ^{14}C activity corrected for the TL between photosynthetic fixation and plant residue input into the soil pool, k the exchange rate constant of the respective C pool, and λ the ^{14}C decay constant (1/8268 a^{-1}). Values for A_i were taken from the atmospheric ^{14}C record of Stuiver et al. (1998) for the period from year 1511 to 1954 and from Levin and Kromer (2004) for the period 1959 to 2004. The period between 1954 and 1959 was linearly interpolated. Data for 2004–2009 were provided by I. Levin ("personal communication").

Carbon mean residence times were calculated according to Eq. 2 by iteratively varying the MRT until it matched the measured ^{14}C activity of the sample. This was done separately for each of the three fractions (roots, cPOM, non-cPOM). Root MRT was directly derived from the bomb model. Mean root MRTs over the eight sites and their respective confidence interval (CI) were taken as TL of the carbon entering the soil when calculating MRT of cPOM and non-cPOM. Another source of error considered in the estimate is the analytical error of the AMS measurement given as one sigma of the pMC value. MRTs of carbon cPOM and non-cPOM were calculated for any possible combination of means and CI (time-lag) or σ (pMC) (i.e. $n = 9$) to derive a more robust error estimate of carbon turnover, shown as mean ($\pm$) one standard error.

Following Leifeld and Fuhrer (2009) and Torn et al. (2009), the flux F of carbon [t C ha^{-1} a^{-1}] through a fraction f (i.e. roots, cPOM, non-cPOM) under steady-state conditions equals the input and was calculated as

$$F_f = 1/\mathrm{MRT}_{\mathrm{fraction}} \cdot \mathrm{poolsize}_{\mathrm{fraction}} \tag{3}$$

with $\mathrm{poolsize}_{\mathrm{fraction}}$ in [t C ha^{-1}], yielding the total flux F_t through the whole soil as the sum of the single fluxes i

$$F_t = \sum F_{fi} \tag{4}$$

and the corresponding MRT [a] for SOC 0–10 cm

$$\mathrm{MRT}_{\mathrm{SOC}} = \mathrm{SOC}_{0-20}/F_t \tag{5}$$

The flux through the root biomass was calculated according to Eqs. 3, 4 but not added to the flux through the whole soil. Mathematically, calculation of bulk soil MRTs based on bulk soil radiocarbon content is possible with the same formula but implicitly assumes kinetics in accordance with a single-pool soil carbon turnover model. Such an assumption violates the evidence of fractions being transformed at different rates and may overestimate soil carbon turnover times (e.g. Trumbore 1997). Therefore, MRTs using two or more fractions of different MRT provide a better approximation of bulk carbon soil dynamics (Leifeld and Fuhrer 2009; Budge et al. 2011).

Roots

The four replicates from each of the eight sites sampled in 2009 were weighed, thoroughly sieved field-moist over a 2 mm mesh and coarse roots were separated. Finer roots in material passing the sieve was hand-picked using a pair of tweezers and combined with the first batch. All roots were carefully washed, freeze–dried, and chopped. Replicates were analyzed separately for C, N, pH (2:1 in water) as above but were bulked for ^{14}C analysis. For decomposition experiments, 0.8 g freeze–dried chopped roots ($n = 32$) were mixed with 19.2 g purified quartz sand and inoculated with 4.2 ml solution inoculum (5.9 mg DOC L^{-1}; extracted from fresh soil from the same site) to reach 60% maximum water holding capacity. Six blanks without roots were run in parallel. Samples were incubated in closed jars containing NaOH at 20°C for 3 weeks and respiration was measured every 3 days by back-titration of the remaining alkalinity.

Statistics

Errors of replicates are given as standard error of the mean (SE). Correlation between parameters was tested using Pearson's correlation coefficient and significant relationships are marked with the respective error probability P. Quantitative effects of pH on carbon mean residence times were studied by ordinary least squares regression and the coefficient of determination. Regression statistics includes standard errors of regression coefficients and CI of the regression line. A t test was applied to test differences in carbon MRT and inputs between groups of different soil acidity. All statistics was calculated using Statistica 9.1, StatSoft Inc., USA.

Results

Soil properties and soil pH relationship to OM and vegetation

All samples were of similar texture (see Table 1) and mineralogical composition. The X-ray diffractograms of the samples were almost identical (data not shown). XRD analysis revealed that the <63 μm fraction was dominated by quartz, plagioclase, K-feldspar, mica, chlorite and actinolite. Minor phases were epidote, rutile, titanite and mixed-layered clay minerals. Major phyllosilicate phases were mica (biotite, muscovite, illite), chlorite, subordinate hydrobiotite (regularly interstratified mica-vermiculite), and interstratified mica-smectite. Some vermiculite was also present. HIV (hydroxy-interlayered vermiculite) and HIS (hydroxy-interlayered smectite) could not be distinguished individually. All samples contained some oxyhydroxides. Among them, lepidocrocite and traces of gibbsite could be identified.

The specific surface area of the fraction <0.63 μm averaged 9.8 (±0.5) $m^2\ g^{-1}$ (NaOCl) and was not related to pH either before or after oxidative treatment. From total element contents (XRF) only total Mg significantly correlated with pH ($r = 0.86$, $P < 0.01$) (see supplementary material). Extractable Ca and Mg was highly positively correlated with soil pH ($r = 0.97$ and 0.91, $P < 0.001$ and $P < 0.01$, respectively) (see supplementary material).

Most OM characteristics of vegetation and soil were not related to soil pH (Table 1). Soil pH affected neither the total amount of SOC or roots, nor the composition, in terms of distribution among soil fractions or C/N ratios (soil or roots). In addition, root degradability, as measured in the incubation experiment, and root mean residence times did not scale with pH. Root pH, however, significantly increased with increasing soil pH but was offset by almost two pH units. Aboveground biomass highly significantly increased with increasing pH and vegetation composition also responded to pH with an increase in the fraction of forbs ($r = 0.71$, $P < 0.05$), whereas the fraction of sedges revealed the opposite pattern ($r = -0.71$, $P < 0.05$). The $\delta^{15}N$ of non-cPOM significantly declined with pH.

Mean residence time of soil fractions and roots

Coarse particulate organic carbon (cPOC) turned over at decadal timescales whereas MRTs of non-cPOC were 1.4–2.9 times longer. MRT of both fractions significantly increased with soil acidity (Fig. 1). However, the slopes of the corresponding regression lines differed significantly. A one pH-unit acidification caused MRT of cPOC to increase by 22% while the

Table 1 Soil and biomass characteristics along the pH gradient

Soil pH	3.9 (< 0.1)	4.7 (0.1)	4.7 (< 0.1)	4.9 (0.1)	5.3 (0.1)	5.5 (0.1)	5.7 (< 0.1)	5.9 (< 0.1)	*r*
Clay (mg g^{-1})	290	290	330	260	310	310	340	310	n.s.
Sand (mg g^{-1})	350	360	270	370	310	300	290	320	n.s.
SOC (%)	12.8 (0.9)	12.8 (1.4)	13.5 (1.0)	10.6 (1.3)	8.5 (0.3)	9.7 (0.5)	11.6 (0.8)	10.1 (0.2)	n.s.
SOC (kg m^{-2})	5.42 (0.46)	6.09 (0.48)	4.86 (0.29)	5.25 (0.61)	5.00 (0.11)	5.03 (0.37)	4.54 (0.69)	4.75 (0.33)	n.s.
C/N soil	13.0 (0.34)	15.3 (0.78)	13.2 (0.11)	14.2 (0.72)	11.7 (0.15)	12.0 (0.15)	11.7 (0.20)	12.1 (0.13)	n.s.
cPOC/SOC	0.26	0.31	0.31	0.23	0.23	0.24	0.18	0.22	n.s.
C/N cPOM	17.3	19.9	14.9	19.6	20.5	18.0	17.1	20.8	n.s.
Root-C (kg m^{-2})	0.45 (0.10)	0.58 (0.07)	0.33 (0.10)	0.73 (0.32)	0.27 (0.07)	0.3 (0.04)	0.29 (0.05)	0.43 (0.13)	n.s.
Root biom. (kg m^{-2})	1.02 (0.24)	1.24 (0.17)	0.71 (0.20)	1.55 (0.65)	0.64 (0.20)	0.66 (0.10)	0.65 (0.13)	1.01 (0.34)	n.s.
Root pH	5.22 (0.03)	4.94 (0.04)	5.14 (0.04)	5.18 (0.01)	5.34 (0.02)	5.42 (0.02)	5.68 (< 0.01)	5.70 (0.01)	0.77*
C/N roots	32.2 (1.9)	47.6 (3.1)	48.0 (6.5)	46.2 (4.9)	34.3 (2.2)	34.5 (3.2)	42.8 (6.0)	52.4 (5.1)	n.s.
Root decay ($d^{-1} \times 1{,}000$)	7.1 (0.4)	6.9 (0.1)	7.3 (0.1)	6.7 (0.5)	7.0 (0.3)	6.9 (0.3)	7.7 (0.2)	6.1 (0.1)	n.s.
Root MRT (years)	7.7	9.2	8.6	17.0	6.1	7.4	7.1	6.8	n.s.
Abovegrd. biomass (g m^{-2})	74.2	109.5	105.0	110.5	126.1	140.9	147.3	124.3	0.90**
$\delta^{15}N$ cPOM (‰)	2.1	0.3	1.2	−0.6	0.2	1.4	1.5	0.8	n.s.
$\delta^{15}N$ non-cPOM (‰)	4.7	4.1	3.8	2.2	2.0	2.8	2.7	2.4	−0.77*
pMC SOC	103.9	104.3	105.8	104.7	110.4	107.3	106.5	109.6	n.d.
pMC cPOC	108.8	109.7	110.0	110.9	113.0	111.1	111.3	111.6	n.d.
pMC non-cPOC	102.1	101.9	103.8	102.9	109.6	106.0	105.4	109.1	n.d.

Errors in parenthesis are one SE. Last column shows correlation coefficient to variable ‘soil pH’

n.s. Non significant, *n.d.* not determined, *pMC* percent modern carbon

Asterisks denote significant relationship ($P < 0.05$)

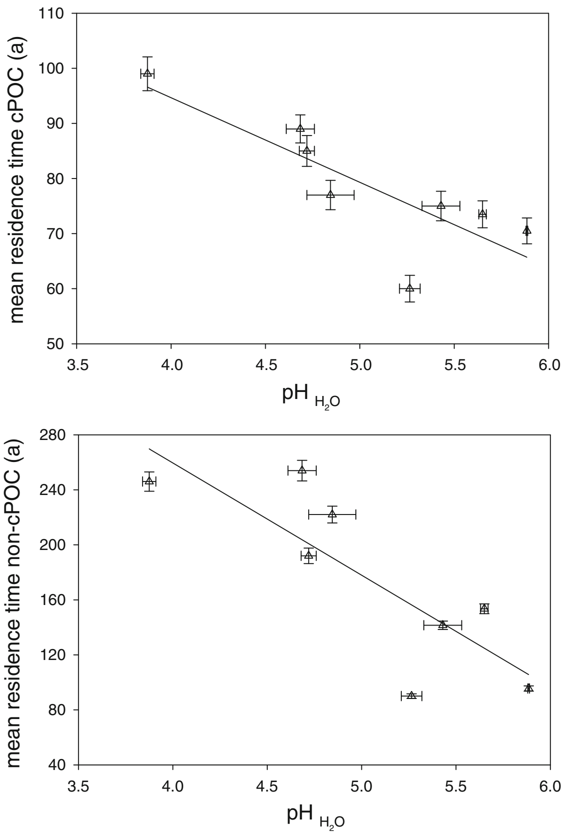

Fig. 1 Mean residence time of cPOC (*top*) and non-cPOC (*bottom*) along the pH gradient and corresponding regression line. Regression equations (±1 SE): MRT cPOC = 156 (±22) − 15.4 (±4.4) * pH (R2 = 0.68, $P = 0.012$); MRT non-cPOC = 586 (±118) − 82 (±24) * pH (R2 = 0.67, $P = 0.013$)

same pH difference caused MRT of non-cPOC to increase by 86%. The result indicates that MRT of non-cPOC responded more sensitively to soil acidity than MRT of cPOC (Fig. 2). In other words, MRT of non-cPOC was on average 2.6 (±0.15) times larger than MRT cPOC below pH 4, whereas it was only 1.7 (±0.17) times larger above pH 4 ($P < 0.01$, *t* test). Figure 2 also reveals that a different pH effect on the two soil fractions only occurred at pH below 6.1 (point of intersection). The mean age of root biomass was 8.7 (±1.2) years. Soil pH has no effect on the mean residence time of carbon in roots (Table 1).

Both the difference in MRT and in $\delta^{15}N$ between non-cPOM and cPOM (Table 1) were negatively related to soil pH ($r = -0.70$, $P = 0.053$ and $r = -0.79$, $P < 0.05$, respectively). A greater age of non-cPOM relative to cPOM thus coincided with a stronger

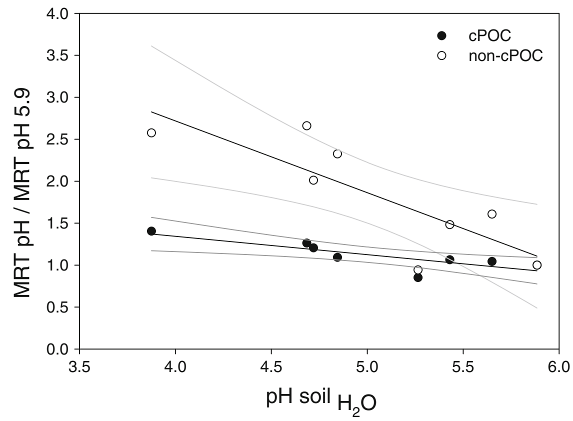

Fig. 2 Mean residence times (MRT) of cPOC and non-cPOC at different pH values relative to MRT at pH 5.9. *Light grey lines* 95% confidence intervals of the regression line. Point of intersection is at pH 6.1. The regression for cPOC is 2.21 (±0.31) − 0.22 (±0.06) pH (±1 SE) and for non-cPOC 6.14 (±1.24) − 0.86 (±0.24) pH. Slopes are significantly different

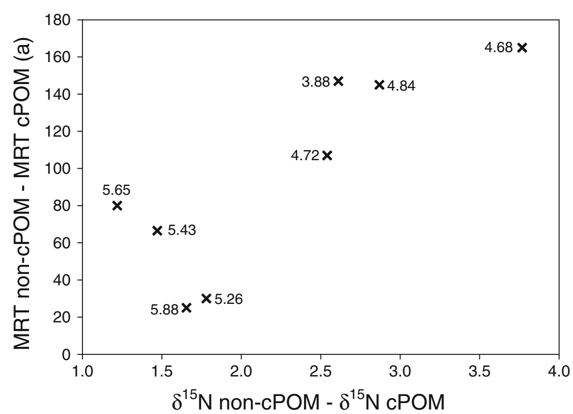

Fig. 3 Difference in $\delta^{15}N$ (non-cPOM–cPOM) relative to difference in carbon mean residence time (non-cPOM–cPOM). *Numbers next to symbols* pH value

enrichment of ^{15}N in that fraction (Fig. 3). The relationship in Fig. 3 was significantly positive ($r = 0.83$, $P < 0.05$).

Carbon flux through soil components

Determination of carbon mass in roots, non-cPOM, cPOM, and bulk soil together with their respective mean residence times allowed calculation of annual carbon fluxes through the various belowground fractions (Fig. 4). At pH below 5.0, the carbon flux through cPOC and non-cPOC fractions was similar

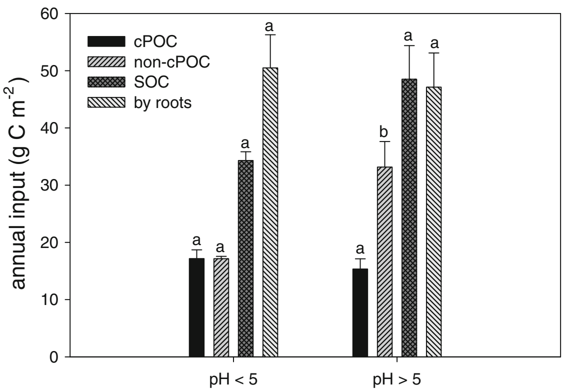

Fig. 4 Annual carbon input to cPOC, non-cPOC, SOC and carbon delivered by root turnover below and above a soil pH of 5.0. *Different letters* significant difference between pH class ($P < 0.05$, *t* test). *Error bars* 1 SE

whereas above pH 5.0, significantly more carbon annually passed through the non-cPOC fraction. In contrast, carbon input delivered to the soil by root turnover was independent of pH.

Discussion

Carbon mean residence times and accrual of cPOM

We found decadal to centennial carbon mean residence times in soil of our subalpine grassland pH gradient. Such long mean residence times are in line with previous studies on subalpine and alpine grasslands (Budge et al. 2011; Leifeld et al. 2009; Neff et al. 2002; Wang et al. 2005). As a matter of principle this attribute may be related to factors controlling soil OM turnover, such as low temperatures, typical for sites at the treeline. The pH dependency of many soil exoenzymes (Sinsabaugh et al. 2008) may be a major mechanism behind the observed relationship between SOC turnover and pH. When low temperature coincides with acidic soil, a frequent combination in mountain regions of humid climates, these two factors act in concert. In addition to temperature and pH, smaller availabilities of Ca and Mg at low pH may limit the overall microbial activity in our soil.

High proportions of cPOM of on average 25% were indicated in this study and seem typical for subalpine and alpine environments (Budge et al. 2011; Leifeld et al. 2009; Neff et al. 2002; Wang et al. 2008). Primarily this pattern might be related to a higher contribution of roots to belowground SOM, as compared to temperate soils (Leifeld et al. 2009), i.e. it might reflect pathways of carbon input. A high proportion of cPOM may also be indicative for factors that affect turnover rates of cPOM in a different way than those affecting turnover rates of non-cPOM because otherwise total carbon stocks, but not the distribution of carbon among fractions, would differ. In previous work (Leifeld et al. 2009), temperature was not found to act differently on cPOM relative to non-cPOM turnover along a grassland elevation gradient. We argue that the observed accrual of cPOM in cold grassland soil is also not caused by direct effects of soil acidity on its decomposition as cPOM content did not scale with pH and low pH was more limiting for the turnover of non-cPOM. The proportion of cPOM to SOM would thus be expected to be maximum at high pH because of the relatively stronger stimulation of non-cPOM decomposition. Hence, high cPOM content in cold grasslands may be mainly driven by other mechanisms such as (i) a higher contribution of roots to belowground inputs, (ii) vegetation-induced poor litter qualities as compared to warmer and fertilized, less acidic sites, and (iii) subsequent preferential feeding by macro-decomposers and shifts in microbial decomposer communities (Eskelinen et al. 2009; Seeber et al. 2009).

Differential response of belowground carbon fractions dynamics to pH

The most prominent observation was the differential effect of pH on turnover of the various belowground carbon fractions under otherwise similar environmental conditions. The differential pH effect on cPOC versus non-cPOC turnover may be explained by two factors. First, the higher pH maintained by roots may attenuate pH limitation on enzyme activities. Root pH varied by only 0.7 units whereas soil pH varied by 2 units. Considering that roots are the main source for cPOM, cPOM turnover rate may benefit less from higher soil pH than non-cPOM because of a higher pH of its feedstock. Therefore, soil pH seems to be an unreliable predictor for pH controls on belowground plant residue decomposition. The stability of root pH across the soil pH gradient may also be one reason for the small variability in root turnover. Additionally, root quality seems largely unaffected by soil pH or vegetation community as both root C/N and root degradability, in the incubation experiment, revealed no trend across sites despite marked differences in vegetation composition and productivity. Second, a relatively strong reduction of non-cPOM turnover below pH 5 may be related to the contribution of mineral associated OM as a potentially stable component of our non-cPOM fraction and the availability and nature of soluble OM as a potentially labile component of our non-cPOM fraction. Because OM solubility, inter alia, depends on its surface charge density, it typically correlates positively with soil solution pH (Kalbitz et al. 2000), supporting a larger microbial availability at higher pH. In addition, at pH < 5 various organic compounds can intercalate into interlayer spaces of 2:1 phyllosilicates, an effective SOM stabilization mechanism, because their degree of dissociation is small (von Lützow et al 2006). Furthermore, complexation of SOM by reactive inorganic hydroxyls via ligand exchange, another powerful stabilization mechanism, usually increases with decreasing pH as it is limited to protonated hydroxyl groups (Kaiser and Guggenberger 2007; von Lützow et al. 2006). Mechanisms related to the nature, and thus inherent degradability, of the substrate may exert additional control on mean residence times. Adsorptive mineral association is selective to the nature of the organic molecule (Kalbitz et al. 2000). The molecular composition is partially driven by the vegetation community which was strongly graded along pH in our case (see site description). Co-precipitation of dissolved OM (DOM) by aluminium, another proposed stabilization mechanism in acid soil, tended to be selective and preferential for compounds high in aromaticity but low in N in samples from a forest soil (Scheel et al. 2007). In the latter study, co-precipitation was shown to be greater at pH 3.8 versus pH 4.5 and DOM mineralization, and thus turnover, was higher at pH 4.5 which is in line with our results. Together, these stabilizing mechanisms may act specifically on the turnover of non-cPOM which includes mineral-associated OM, reducing the exchange rate and thus the microbial availability of OM at low pH. This is in line with the much longer MRT of non-cPOM in soil of greater acidity. Concentrations and thus availability of DOM, however, may be higher at low pH in contrast to its genuine solubility due to a decline in the degree of metal–organic complexation with increasing acidity (proton competition; Guggenberger et al. 1994). Our data indicate that the latter mechanism may be of minor importance but that a high pH supports DOM availability and thus turnover.

Differences in soil pH often go along with differences in soil mineralogy and the latter exerts control on the stabilization of mineral associated OM (Denef et al. 2004; Mikutta et al. 2009). However, there is no indication for differences in soil texture, mineralogy, bulk elemental composition, or the surface area of the fine soil fraction across our pH gradient. We therefore consider possible effects induced by differences in soil mineralogy on altering OM turnover rates to be negligible at these sites.

^{15}N enrichment as a function of soil pH

A longer MRT of non-cPOM coincided with a stronger ^{15}N enrichment in non-cPOM relative to cPOM. This enrichment is most probably a result of isotope discrimination processes along microbial transformation pathways and corresponds to previous studies showing that non-cPOM and mineral-associated OM is microbially more transformed than POM (e.g. Conen et al. 2008; Kramer et al. 2003; Tiessen et al. 1984). Interestingly, the ^{15}N signature of cPOM did not change with pH whereas that of non-cPOM increased with declining pH, i.e. the degree of microbial transformation of stabilized OM was larger in acidic soil. At the same time, we calculate significantly smaller carbon inputs into the non-cPOM fraction at pH below 5. The difference in input was about 14 g C m^{-2} a^{-1} between sites below and above pH 5, and corresponded well to the difference in aboveground productivity of about 35 g dry matter m^{-2} a^{-1}. Hence the higher delivery rate by the vegetation caused by the larger aboveground productivity, at higher pH, may be one reason behind the finding that organic matter recovered in the non-cPOM fraction at higher pH had a $\delta^{15}N$ signature more closely to that of plants. The nature of the substrate may also play a role in OM stabilization, resulting in a pH-dependency of $\delta^{15}N$ in the non-cPOM fraction. The various soil organic N pools differ substantially in their isotopic signature (Yano et al. 2010) and a preferential adsorption of any of these compound classes at low pH might result in a systematic shift in $\delta^{15}N$ of non-cPOM. With our data set we cannot unravel the mechanisms behind the isotopic systematics but findings point toward a differentiation in the

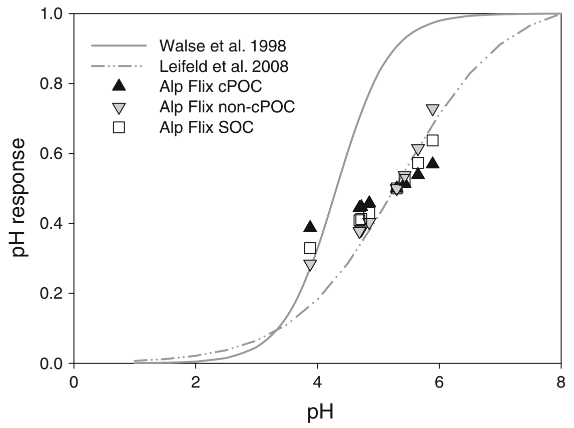

Fig. 5 Comparison of pH response functions for litter decomposition (Walse et al. 1998), cPOC turnover (Leifeld et al. 2008), and for bulk soil carbon, cPOC and non-cPOC from this study. The midpoint of the sigmoid (i.e. pH response = 0.5) was assumed to be the same as in the function of Leifeld et al. (2008)

type of molecules involved, as well as in rates of carbon delivery and mechanisms and strengths of mineral association considering that non-cPOM also includes mineral-associated OM.

Conclusions

A comparison of pH response factors from this study with previous work confirms a strong pH-dependency of soil carbon turnover rate (Fig. 5). Eskelinen et al. (2009) argued soil pH to be the ultimate factor driving vegetation and microbial community patterns in tundra soil. We add that pH is a key driver for the turnover of organic matter in cold grassland soil because the previously stated strong dependency of turnover rates on pH has now been quantified and confirmed under long-term steady-state field conditions. We argue that soil pH should be an integrative part of global carbon and nitrogen turnover modelling. Soil acidity exerts stronger control on turnover of older non-cPOM than on residue decomposition, albeit the effect is significant in both cases. This differential effect is related to the pH of the corresponding feedstock, or the solution in its vicinity, and to pH-dependent stabilization of mineral associated OM.

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