Atmospheric N deposition causes carbon balance gains in a seven year field experiment in subalpine grassland

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Abstract. Air pollution agents interact when affecting biological sinks for atmospheric CO_2, e.g. the soil organic carbon (SOC) content of grassland ecosystems. Factors favoring plant productivity, like atmospheric N deposition, are usually considered to favor SOC storage. In a seven year experiment in subalpine grassland under N- and O_3-deposition treatment, we examined C-fluxes and -pools. Total N deposition was 4, 9, 14, 29 and 54 kg N ha^{-1} yr^{-1} (N4, N9, etc.), annual mean
10   phytotoxic O_3 dose was 49, 65 and 89 mmol m^{-2} projected leaf area. We hypothesized that SOC of this mature ecosystem would not change in control treatments, but that effects of air pollutants on plant yield, net ecosystem productivity (NEP) and SOC content would develop in parallel, leading to SOC content to increase with N deposition. In the control treatment SOC increased significantly by 9% in seven years. Cumulative plant yield showed a highly significant N effect (+38% in N54), but no O_3-effect. Cumulative NEP did show a strong, yet statistically insignificant, hump shaped response pattern to N
15   deposition with a +62% increase in N14, and only +39% increase in N54. SOC had a similar response to N, with highest gains at intermediate N deposition rates (9 and 14 kg N ha^{-1} yr^{-1}), suggesting an unimodal response, too. We assume the strong, pollutant-independent soil C sink developed as a consequence of the management change from grazing to cutting. The non-parallel response of SOC and NEP compared to plant yield under N deposition is likely the result of increased respiratory SOC losses, following mitigated microbial N-limitation or priming effects, and a shift in plant C allocation
20   leading to smaller C input from roots.

Keywords: Carbon sequestration, nitrogen deposition, tropospheric ozone, grassland, mountain, ecosystem CO_2 fluxes, SOC, R_{eco}, GPP, NEP

1 Introduction

25   Biological sinks for atmospheric CO_2, including grassland soils, substantially modulate the increase rate of this greenhouse gas (Sitch et al., 2008) and are also viewed as tools for greenhouse gas increase mitigation (Conant, 2010). Grassland soils contain 661 Pg C, equivalent c. 28% of total global soil C (excluding wetlands and frozen soils (Jobbágy and Jackson, 2000)) or >80% of C contained in the atmosphere. Climate change effects were predicted to decrease European grassland soil organic carbon (SOC) stocks by 6-10% during the 21st century (Smith et al., 2005). It is thus necessary to address the

factors that determine the ecosystem carbon (C) sink properties, including air pollution agents, which interact to affect C sinks in grasslands.

Atmospheric N deposition exceeds critical loads in European lowlands, thus increasing ecosystem productivity and affecting biodiversity (Bobbink et al., 2010; Phoenix et al., 2012). So far, N deposition is much lower (c. 5 kg ha^{-1} yr^{-1}) at high altitude

5   and in inner-alpine valleys (Rihm and Kurz, 2001) like our study site. Also, tropospheric O_3 concentrations have reached levels that affect plant growth (Fuhrer et al., 1997; Ashmore, 2005; Volk et al., 2006) and least conservative estimates predict mean annual O_3 background concentrations to rise to 84 ppb by 2100 (Vingarzan, 2004; Derwent et al., 2007). Ozone concentrations are particularly high at high altitude sites, due to reduced nocturnal degradation and deposition, and the natural gradient under free-tropospheric conditions (Chevalier et al., 2007).

10 C storage in ecosystems begins with plant growth. It is thus reasonable to expect that increased plant growth under increased N deposition has a similarly positive effect on ecosystem C storage (Vitousek et al., 1997). For example Ammann et al. (2009) found higher C sequestration in an intensively managed, high N input (c. 240 kg N ha^{-1} yr^{-1}) grassland, compared to an extensively managed field (no N fertilizer input) and in forests atmospheric N deposition showed a strong positive correlation with C sequestration (Magnani et al., 2007). But additional N input (together with CO_2 enrichment) also has the

15 potential to hinder C sequestration by favoring the decomposition rate of soil organic matter more compared to plant productivity (Soussana and Hartwig, 1996). Meanwhile, a growing awareness of the highly dynamic and complex interactions of both climate parameters (e.g. water availability and temperature) and multiple pollution factors (e.g. N and O_3) has developed. Such scenarios lead to assume that the C sink of terrestrial ecosystems will show a much smaller growth, or even turn into a substantial source of atmospheric CO_2 (Lu et al., 2011; Heimann and Reichstein, 2008).

20 In the past, the scale for N input was often adopted from agronomic practices. Experimentalists used high, single dose N input treatments, and also extensive analyses did not differentiate effects of high and low N input doses (e.g. Liu and Greaver, 2010), summarizing N inputs from 10 to 650 kg N ha^{-1} yr^{-1}). But in their meta-analysis of fertilization experiments (24-600 kg N ha^{-1} yr^{-1} treatment range) Knorr et al. (2005) concluded that $<20 \times$ ambient N deposition or low-quality (high-lignin) litter inhibited decomposition. On the other hand, $>20 \times$ ambient N deposition or high-quality (low-lignin) litter

25 stimulated decomposition, suggesting reduced ecosystem C storage in high N deposition environments. Usually, natural and semi-natural vegetation, including grasslands, does not receive management similar to such experimental conditions. Instead, atmospheric N input comes in a variety of doses that are very low by agricultural standards, combined with additional environmental agents like O_3 pollution. To make matters more complicated, different weather conditions in every growing season have the potential to alter quality and quantity of the individual pollutant effects.

30 Despite their applicability for the prediction of C sink properties under changing environmental conditions, low input dose, multilevel N deposition and multifactor experiments focusing on the carbon budget are rare in (semi-) natural ecosystems. Those available, usually studied one or more potentially beneficial atmospheric inputs, e.g. multi-level N deposition, multi-nutrient application (e.g. Fornara and Tilman, 2012; Fornara et al., 2013) or N $\times$ CO_2 interactions.

An earlier analysis in the same project, applying a fully factorial, multilevel N × O_3 deposition treatment, had shown plant dry matter yield to increase consistently with rising N deposition rates (Volk et al., 2014), but there was no O_3 effect or N × O_3 interaction. A net ecosystem CO_2 balance (net ecosystem productivity, NEP), that was parameterized for an exceptionally warm, single year, had shown C losses at all treatment levels and combinations, but the largest C loss coincided with the

5   highest N deposition treatment applied (Volk et al., 2011). Meanwhile, the full meteorological record for NEP parameterization, belowground yield data and SOC analysis from the destructive final harvest of the experimental plots is available. In the study presented here, we investigate the seven year impact of N and O_3 deposition on the grassland ecosystem C budget, from CO_2 exchange via plant productivity to SOC. We hypothesized that

I) 'Time effects', most importantly representing the interannual weather variability, cause the annual NEP to be positively

10   correlated with temperature, reflecting the positive effect of warmer growing seasons on aboveground plant yield in this cold, high altitude environment. SOC content was expected to remain unchanged over seven years. The grassland developed under a low intensity management that was unaltered for decades, if not centuries. We therefore considered SOC to be in steady state in a mid- and long-term perspective.

II) 'Air pollutant effects' (O_3 and N deposition) cause both NEP and SOC to respond analogous to plant productivity. This

15   implies a consistently positive response to rising N input and a negative or zero response to increased O_3 deposition.

The study intends to improve the understanding of the C balance of extensively used, semi-natural grassland under realistic air pollution scenarios, typical in the context of a global change scenario.

2 Material and methods

2.1 Experimental site and meteorological conditions

20   The experiment was located at 1990 m a. s. l., on a grassland plateau in the Central Alps (46° 32' N, 9° 39' E; Alp Flix, Sur, Canton Grisons, Switzerland). The natural tree line is at ~2200 m a. s. l.. Low-intensity management prior to the experiment (approximately 60 yrs.) was 3–4 weeks of cattle grazing at ~1.3 livestock units ha^{-1}. No manure or fertilizer was applied. Winters are cold with permanent snow cover from December until April. Annual mean temperature during the seven year experiment was 1.1 °C, with an April to October mean of 6.2 °C. Mean April to October precipitation was 853 mm.

25   Background O_3 hourly mean concentration for the growing period varied from 40 to 50 ppb. Very small diurnal and seasonal fluctuations are typical for this high-elevation site. Background N deposition - calculated from concentration measurements in air, rainwater and snow - amounts to <4 kg N ha^{-1} yr^{-1} (Bassin et al., 2007). A comprehensive description of the physical environment can be found in (Volk et al., 2014).

2.1 Experimental plots and treatment application

30   Plots consisted of 180 intact turf monoliths (L × W × H = 30 × 40 × 22 cm) that were excavated in the fall of 2003, placed in drained plastic boxes and randomly assigned to treatment combinations. Groups of 20 were placed in shallow pits, flush with

the surrounding surface in the center of nine O_3 fumigation rings. To minimize confounding effects of microclimatic differences between rings, monoliths were rearranged annually between the fumigation rings, but kept within their respective O_3 treatment. Both the O_3 and N treatments were applied during the snow-free period from approximately May to October.

5 Accounting for 4 kg N ha^{-1} yr^{-1} background deposition, total N loads in the five different treatments were 4 (control), 9, 14, 29, and 54 kg N ha^{-1} yr^{-1}. They are identified below as $N4_{control}$, N9, N14, N29, and N54, respectively. The extra N amounts were applied as bi-weekly fractions of an ammonium nitrate (NH_4NO_3) solution in 200 ml of well water per monolith. $N4_{control}$ monoliths received only water. Each N level was replicated four times in each fumigation ring.

The free-air ozone fumigation system (for details see Volk et al., 2003) had a control treatment (ambient $[O_3]$, termed O_3 control) and two O_3 enrichment levels that achieved 1.4 × and 1.7 × ambient concentration (O_3+ and O_3++, respectively).
10 Each treatment level was randomly assigned to three out of the total of nine fumigation rings. Ozone was continuously sampled at canopy height in the middle of each ring and measured with Dasibi 1008 series UV analyzers (Dasibi, Glendale CA, USA).

Annual mean accumulated exposure over a threshold of 40 ppb O_3 (AOT40) was 9.7, 21.0 and 38.9 ppm.h and phytotoxic ozone dose over the threshold of 0 mmol m^{-2} (POD0) was 48.7, 64.8 and 89.1 mmol O_3 m^{-2} projected leaf area in O_3 control,
15 O_3+ and O_3++, respectively (Volk et al., 2014). For details on the O_3 treatment please refer to Volk et al. (2003).

2.2 Plant yield C content

Aboveground plant biomass was cut annually at peak canopy development (end of July), at 2 cm height. The harvested material was oven dried and weighed to yield dry matter mass (DM). For details please refer to Bassin et al. (2007). Belowground root biomass was assessed from soil cores covering a subset of the monoliths. DM masses were expressed as g
20 C m^{-2} based on plant C concentration measurements of key species (Bassin et al., 2015). Masses of four plots each were combined and averaged to match the lumping rules developed for soil sampling (compare below). Tests for effects of N- and O_3 deposition on plant C concentration yielded no results and a common value was assumed.

2.3 Net ecosystem productivity

Net ecosystem CO_2 exchange (NEE) was measured in 17 day + night campaigns, between end of May and end of October
25 2010 (the seventh treatment year), covering the complete growing season. The subset of 27 monoliths used covered all three O_3 treatments and three out of five N deposition treatments ($N4_{control}$, N14 and N54). The method utilizes a dynamic CO_2 concentration, non-flow-through, transparent acrylic chamber, fit to cover the whole grassland monolith including a fully developed canopy. Reduced assimilation or respiration due to chamber effects (CO_2 substrate depletion, overheating, reduced diffusion gradient) could be safely excluded during the short measurement (2 min. per monolith).
30 We parameterized seven year cumulative NEP CO_2 balance (NEP_{cum}) for the 2004-2010 period, based on NEE CO_2 flux data from 2010. We used global radiation (GR) and soil temperature at 5 cm depth (T_{soil}) to model gross primary productivity (GPP) and ecosystem respiration (R_{eco}), respectively. All methods are equivalent to Volk et al. (2011), except for an

optimized exponential function for R_{eco} after Lloyd and Taylor (1994) and Ammann et al. (2007). System respiration substrate limitation was accounted for based on reduced respiration observed in winter in mountain grassland (Galvagno et al., 2013). Accordingly, temperature normalized R_{eco} during the snow covered period was modelled to drop linearly to 50% of the last fall measurement until Dec. 31st. From Jan. 1st a linear rise from 50% of the first spring measurement to 100% at the date of this first measurement was assumed.

2.4 Soil organic carbon

SOC data presented here derive from all 180 monoliths, covering all five N- and three O_3-treatment levels. At the setup of the experiment in October 2003 soil from 0-10 cm and 10-20 cm depth was cut from the walls of the pits that resulted from the excavation of the monoliths. The soil density of these samples is not known. Seven years later, in October 2010, two cylindrical soil cores per monolith were sampled from the surface to 10 cm and from 10-20 cm depth. All samples were dried and sieved (2 mm). For analysis sieved samples from four monoliths each, receiving the same treatment combination, were lumped to get a total of 45 samples per sampling depth.

We measured soil organic C and N contents by elemental analysis (oxidation of $C-CO_2$ and $N-NO_2$ in an O_2 stream and subsequent reduction of NO_2-N_2 by a copper-tungsten granule). Separation of CO_2 and N_2 was accomplished by GC-TCD and quantification using acetanilid as an external standard (Hekatech Euro EA 3000, Wegberg, Germany). Samples were free of carbonate, so total C equals organic C. All soil C stock values (kg C m^{-2}) are based on density values established using the 2010 samples.

2.5 Statistics

Effects of treatments were tested in a split plot analysis using a linear mixed effect model (Pinheiro and Bates, 1996) with O_3 at the main plot level and N at the sub-plot level. For SOC concentration (45 samples), plant C (45 samples) and NEP_{cum} (27 samples) replication was n = 3 for O_3 on three treatment levels and n = 9 for N on five (SOC, plant C) or three (NEP) treatment levels. Block entered the model as class variable, whereas O_3 and N were continuous variables. The O_3 treatment was quantified as the seven years cumulative POD0. Inference on the significance of variables and selected interactions was made using likelihood ratio tests (Burnham and Anderson, 2002). Data were Box-Cox-transformed when necessary. All analyses were carried out by means of the statistics software R (R Development Core Team, 2011) using the packages nlme, nortest, lattice and car. Probability of equal means for SOC concentrations in 2003 vs. 2010 was tested using a two-sided, paired Student's t-test on samples from the control treatments. Values in the text and the figures are means ±1 standard error.

3 Results

3.1 Cumulative plant C yield

3.1.1 Time effects

In the control treatment, the mean plant C contained in total above- and belowground biomass was 591 ±60.3 g C m^{-2}. Of
this plant material an annual mean of 74.9 ±7.64 g C m^{-2} yr^{-1} was exported with the grass harvest (Fig. 1). Thus, seven years
cumulative aboveground plant C yield (Yield$_{cum}$) in control plots is equivalent to 524 g C m^{-2}.

3.1.2 Air pollutant effects

Yield$_{cum}$ in seven years showed a highly significant N-effect, but neither an O$_3$-effect nor an N × O$_3$ interaction. The increase
of yield with increasing N deposition was continuous, but not linear, as indicated by the significant N × N interaction (Fig. 2;
Tab. 1). Compared to N4$_{control}$ (509 ±15.4 g C m^{-2}) the maximum Yield$_{cum}$ increase was +38% in the N54 treatment (702
±15.6 g C m^{-2}).

3.2 Net ecosystem productivity

3.2.1 Time effects

In the control treatment the ecosystem CO$_2$ exchange balance for single years ranged from a loss of -14.2 ±30.44 g C m^{-2} in
warm and dry 2006 to a maximum gain of 68.3 ±28.56 g C m^{-2} in cool and moist 2010 (Fig. 1). Linear regression of NEP
over soil temperature yielded a negative correlation with R^2 = 0.82 (P = 0.005). NEP$_{cum}$ for the seven year period was 245
±210.0 g C m^{-2}.

3.2.2 Air pollutant effects

In NEP neither O$_3$ nor N caused statistically significant treatment effects or interactions (Tab. 2). Surprisingly, NEP$_{cum}$ is
smaller under N54-deposition (Fig. 3) compared to the N14 treatment (lumped over O$_3$ treatments: N4$_{control}$ 279 ±89.3 g C m^{-2}, N14 452 ±81.4 g C m^{-2}, N54 387 ±96.0 g C m^{-2}). Owing to the low number of treatment levels (three), statistical testing
for N × N interaction is not meaningful, yet the response of NEP$_{cum}$ to rising N deposition suggests a hump shaped pattern.

3.3 Soil organic carbon

3.3.1 Time effects

From 2003 to 2010 bulk soil C concentration in the 0-20 cm soil layer (control treatments) increased from 61 ±1.9 g kg^{-1} to
67 ±0.6 g kg^{-1} (P = 0.052). The concentration increase was unevenly distributed with +12% in the 0-10 cm layer (2003: 87
±2.5 g kg^{-1}, 2010: 97 ±1.1 g kg^{-1}; P = 0.019) and +3% in the 10-20 cm layer (2003: 36 ±3.4 g kg^{-1}, 2010: 37 ±1.1 g kg^{-1}; *n.s.*;
Fig. 4). The resulting C stock in the 0-20 cm layer grew from 6.9 ±0.18 to 7.5 ±0.02 kg C m^{-2}, equivalent +9% increase. A

resampling campaign (0-10 cm depth) in 2012, at the site where the turf monoliths were excavated in 2003 and where the seasonal grazing had continued since, yielded a bulk soil C concentration of 83 ±1.5 g kg^{-1}, marginally less than the first sampling nine years ago.

3.3.2 Air pollutant effects

5 The N deposition related C gain occurred mostly in the top 10 cm of the soil column (Fig. 5). This soil layer shows a marginally significant N × N interaction (Tab. 3), indicating a hump shaped response function of SOC to N deposition. But no significant effects or interactions were found for N in the lower layer or for O_3 in either upper or lower soil layer. In the 0-10 cm soil layer all SOC stock increases were higher in the N deposition treatments compared to N4$_{control}$. But at low deposition rates (N9, N14) SOC kg m^{-2} increased much further than at high deposition rates (N29, N54) (Fig. 5). Changes in
10 the 10-20 cm layer mirror the upper layer, only there is no gain in N4$_{control}$.

4 Discussion

4.1 Cumulative plant C yield

Because O_3 did not show an even remotely significant effect on either plant C yield, NEP or SOC, here we do not discuss the highly variable responses, observed in the O_3 treated grassland plots.

15 Despite a decreasing N effect, Yield$_{cum}$ (Fig. 2) is monotonically increasing over the whole range of rising N deposition rates. This is a typical response across a range of ecosystems, with the response function depending mostly on the productivity of the system (e.g. Yuan et al., 2006). Surprisingly, the positive N effect on plant C yield translated unevenly into higher C values for the parameters that are directly indicative of the ecosystem C budget (compare below).

4.2 Net ecosystem productivity

20 Despite a substantial gain over seven years, NEP$_{cum}$ suggested smaller C gains than those finally found in SOC. We suspect that a temperature based overestimation of C losses contributed to a small NEP$_{cum}$. In a sensitivity test of R$_{eco}$ a 1°C reduced soil temperature increased NEP$_{cum}$ to 787 ±194.9 g C m^{-2}. Thus, a small error in the temperature parameterization could be one reason for the observed mismatch between NEP$_{cum}$ and SOC content.

GPP parameterization is based on the comparatively small 2010 canopy (cf. yield values Fig. 1) and NEP$_{cum}$ may therefore
25 contain an underestimation of GPP. To explore the potential error, we alternatively scaled GPP linearly with aboveground yield of the respective years (2010 = 100%) and found a NEP$_{cum}$ of 1.61 ±0.23 kg C m^{-2}. This is c. 40% more than the sum of SOC gain (0.6 kg C m^{-2}) plus plant C exported with the harvest (0.5 kg C m^{-2}) and implies that GPP does not grow proportionally with the size of the green canopy.

Although this scaling exercise substantially increased NEP, interannual comparison shows that canopy size and its influence
30 on GPP play only a small role for NEP, compared to the temperature effect on R$_{eco}$. For example two years of very similar

sized canopy showed the maximum NEP in 2010 (68 g C m^{-2}), but also the minimum NEP in 2006 (-14 g C m^{-2}). Also, both negative NEPs (2006 and 2009) coincided with the lowest and the highest annual aboveground yield during the experiment (Fig. 1). Thus, contrary to expectations, no correlation could be found between annual NEP and yield. Instead, the lowest (i.e. most negative) NEPs were found in years with highest mean soil temperature, exactly like Marcolla et al. (2011) report

5 (for air temperature) from a seven year mountain grassland eddy covariance study. In our experiment linear regression of NEP over mean T_{soil} for the respective years results in $R^2 = 0.82$. This made T_{soil} the largest single factor determining whether the system was a C sink or source in a given year.

Under air pollution treatment we found an unimodal/hump shaped response pattern of NEP$_{cum}$ (Fig. 3), but the significance of the N × N interaction is not testable with three treatment levels. We suggest a mechanism that, at low N deposition rates,

10 favors assimilatory plant C gains more and, at high N deposition, favors the antagonistic, respiratory soil C decomposition losses more.

The R$_{eco}$ and GPP data, used for NEP$_{cum}$ parameterization, allowed us to test the above proposition: For cumulative C loss from R$_{eco}$ the N effect in the N14 treatment (N14/N4$_{control}$) is +3.3%, but in the N54 treatment (N54/N4$_{control}$) it rises to +5.8%, equivalent of a factor 1.8 increase. For cumulative C gain from GPP on the other hand, the positive effect in N14 is

15 already +7.3%, but the rise in the N54 treatment is only to +8.1%. This is equivalent to an increase of only factor 1.1 between N14 and N54 treatment level. Thus, the response functions developed differently in assimilatory vs. respiratory processes and GPP is not growing substantially, once a comparatively low N deposition rate is exceeded.

Skinner (2013) reports the same pattern from eddy covariance measurements in a grassland fertilization experiment: In a first five year period NEE yielded C gains under low N (+26 kg N ha^{-1} yr^{-1}), compared to C losses under high N deposition (+83

20 kg N ha^{-1} yr^{-1}) treatment. With substantially increased fertilizer application (low +85, high +217 kg N ha^{-1} yr^{-1}) during the subsequent four year period all NEE balances were negative, indicative of the high N deposition treatment promoting respiration processes more than assimilation processes.

4.3 Soil organic carbon

SOC concentration values observed here are well within the established limits for this vegetation type. In the 0-20 cm layer

25 of 15 grassland soils above 1000 m a.s.l., described by the Swiss National Soil Observatory (Desaules and Dahinden, 2000), SOC ranges from 3.5% to 8.7% (mean 5.4, SD 1.40). In a literature overview Leifeld et al. (2005) calculated a mean of 6.07% (SD 3.31) for >1000 m a.s.l. permanent grassland in Switzerland.

To convert 2003 SOC concentrations measured into SOC stocks, we used the soil density values established in 2010. We assumed the management change did not result in decreased soil density from reduced trampling, because the Leifeld and

30 Fuhrer (2009) study in adjacent plots showed no differences, even 60 years after the meadow/pasture management change. Both grasslands had a ρ = 0.64 ±0.04 for 0-16cm depth, slightly higher but very similar to the ρ = 0.60 ±0.005 we found for 0-20cm soil depth in this study.

The small decrease in SOC concentration under continued seasonal grazing, found at the 2012 resampling of the site where the monoliths came from, showed that the effect seen in the trial must have been a treatment effect or a side effect of the experimental management. We suggest that the newly introduced cutting at only 2.5 cm above the ground represents a more intensive use, compared to selective grazing by cattle, and that this has created a new SOC source. Because plants maintain a
5   functional root/shoot equilibrium (Poorter et al., 2012), the aboveground harvest will cause a proportional root dieback. In the control treatment the mean annual above- plus belowground live plant C content was >500 g C m^{-2}. If all newly introduced soil C (0.6 kg m^{-2}) in seven years derived from solid plant material, an additional 86 g C m^{-2} yr^{-1} or c. 15% of the plant C stock must have been incorporated in the soil, compared to steady state SOC stock conditions. Indeed, a mean of 74.9 $\pm$7.64 g C m^{-2} yr^{-1} was cut and exported with the grass harvest, an amount quite similar to the required annual SOC
10   input, making proportional root dieback a possible source for the belowground C gain. Similarly, from the eight eddy covariance mountain sites of the Carbomont network it was concluded that NEE for pastures was close to zero, while cut meadows accumulated carbon (Berninger et al., 2015). This 'grazed vs. cut' contrast could be equivalent to the long-term Reeder and Schuman (2002) grassland study, with increasing SOC stocks along a gradient of increasing grazing intensity. Further support comes from the Leifeld et al. (2015) study, showing that root median residence time was approximately
15   halved (from 7.3 to 3.8 years), when grassland management intensity rose from 'low' to 'medium'. This intensity change is similar to what the grassland monoliths in our study experienced, when they were transferred from the occasionally grazed pasture into the experimental cutting regime.

Under N deposition air pollution treatment SOC mirrored the response pattern seen in the seven years NEP parameterization. The strong increase at low, but only small increase at high N deposition rates, demands an asymmetric promotion of C
20   accumulation at different N deposition rates. Both soil layers (Fig. 5) show this characteristic pattern, which makes it likely that the same processes are active, only with a lag phase and at a slower rate in the lower layer. This is because the top soil layer has a higher proportion of recently assimilated, labile C, consisting of plant litter, roots, microbial and fungal biomass, as indicated by the $>2 \times$ higher soil C concentration (Fig. 4).

Some studies suggested soil C content to increase by reducing organic matter decomposition following high N deposition,
25   either as a result of abiotic interactions with microbial products or from effects on the decomposer community (Hobbie, 2008). High lignin litter reduced decomposition in Dijkstra et al. (2004), while Waldrop et al. (2004) differentiates accelerated decomposition of easily decomposable litter and reduced decomposition of recalcitrant (high lignin) litter.

But the Park Grass Experiment at Rothamsted, UK (Fornara et al., 2011) shows no change of SOC content in N fertilized plots (96 kg N ha^{-1} yr^{-1}) between 1876 and 2005, indicative of the N-input rate to be beyond the positive C-balance point. In
30   an alpine grassland at Niwot Ridge LTER Neff et al. (2002) found N deposition >100 kg ha^{-1} yr^{-1} to accelerate microbial decomposition of labile C pools, leading to a zero soil C balance despite substantially increased plant C input.

Increasing N doses were also found to decrease soil C content via higher microbial activity. Generally speaking, microbial activity or population size may suffer similar nutrient constraints as plant productivity does (cf. 'Law of the minimum'; von Liebig, 1840). N limitation of soil microbial growth has been suggested based on theoretical considerations (Schimel and

Weintraub, 2003) and Stone et al. (2012) found increased activity of extracellular hydrolytic enzymes from soil microbiota after long-term 25-35 kg ha^{-1} yr^{-1} N deposition in the field. A meta-analysis of 77 grassland studies on N deposition and CO_2 enrichment effects (Sillen and Dieleman, 2012) concluded that 'moderate' N deposition (equivalent to the maximum N deposition in our experiment) was accelerating decomposition processes, thus reducing the soil C storage potential. Likewise Li et al. (2014) report maximum SOC concentrations at intermediate rather than maximum treatment levels after five years of high N input rates (50, 100 and 150 kg N m^{-2} yr^{-1}) in alpine meadows, finding the hump shaped response at generally high input levels.

Also the quality of available C as a source of energy plays an important role. Fontaine et al. (2003) describe a mechanism that may often stand behind substantial soil C losses in the face of increased plant growth: Newly available carbohydrates in the soil create a competitive stimulation of microbial populations that results in strongly increased decomposition rates (priming effect).

But besides decomposition changes of uncertain direction, plant C allocation plays an important role. In an earlier analysis Volk et al. (2014) had found N deposition to change the Shoot/Root ratio (S/R) only marginally between N4$_{control}$ (0.22 ±0.013) and N14 (0.23 ±0.013). But S/R increased quite dramatically in N54 (0.29 ±0.009), equivalent to a 24% smaller root proportion of total biomass. Thus, despite increasing aboveground plant C mass, a strong allocation shift, away from roots in favor of aboveground plant organs, may have led to smaller soil C input from root turnover.

Lacking low dose, multilevel N deposition treatments, cases of tipping response functions could usually not be found as a consequence of the experimental design. But in the 19 year, multi-nutrient experiment described by Fornara et al. (2013), the effect of multiple nutrients may constitute an analogy to the effect of increasing N deposition levels. Smaller C sequestration was found in NP, PK and NPKMg treatments compared to the N only application, while aboveground plant mass grew consistently with nutrient addition (Control < N < NP < NPKMg). This is indirect evidence that a more effective plant fertilization does not necessarily result in higher SOC sequestration, but instead creates a situation where decomposition or allocation changes are more favored than assimilation, at higher or combined nutrient addition treatments, with negative effects on soil C content.

We conclude that annual NEP was negatively correlated with soil temperature, not positively correlated with air temperature like plant yield. Contrary to expectations, soil C concentration in the subalpine grassland substantially increased over time. Thus, as an effect of management change from grazing to cutting, the soil was a strong C sink in the 2003-2010 period. Despite consistently positive responses of aboveground plant yield to increasing N deposition, SOC increased substantially only at low N deposition, but grew less at high deposition rates. NEE measurements suggest, that N deposition caused extra C-losses via higher microbial respiration after mitigation of N limitation or as a consequence of priming, resulting from the increased input of fresh organic plant material. Also plant C-allocation changes may have contributed to reduced soil C input from roots at high N deposition doses. These mechanisms are likely responsible for the hump shaped response of SOC to increasing N deposition. Accordingly, N deposition driven yield increases in low productivity grasslands may not be considered as a valid proxy for parallel ecosystem C-pool increases.

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Table 1

	L-ratio	p-value
Block	10.5	0.005
O_3	0.3	0.582
N	48.2	0.000
$O_3 \times N$	2.1	0.151
$N \times N$	5.4	0.020

Table 1 Split plot analysis of cumulative seven year plant C yield. Effects of block, ozone (O_3), nitrogen (N) and selected interactions.

Table 2

	L-ratio	p-value
Block	1.8	0.410
O_3	0.0	0.859
N	0.7	0.387
$O_3 \times N$	0.0	0.995

Table 2 Split plot analysis of cumulative seven year net ecosystem productivity (NEP). Effects of block, ozone (O_3), nitrogen (N) and $O_3 \times N$ interaction.

Table 3

	L-ratio	p-value
Block	1.36	0.51
O$_3$	0.58	0.45
N	0.25	0.62
O$_3 \times$ N	0.47	0.49
N $\times$ N	2.85	0.09
O$_3 \times$ N $\times$ N	0.02	0.88

Table 3 Split plot analysis of change of soil organic carbon concentration (0-10 cm layer) between 2003 and 2010. Effects of

block, ozone (O$_3$), nitrogen (N) and selected interactions.

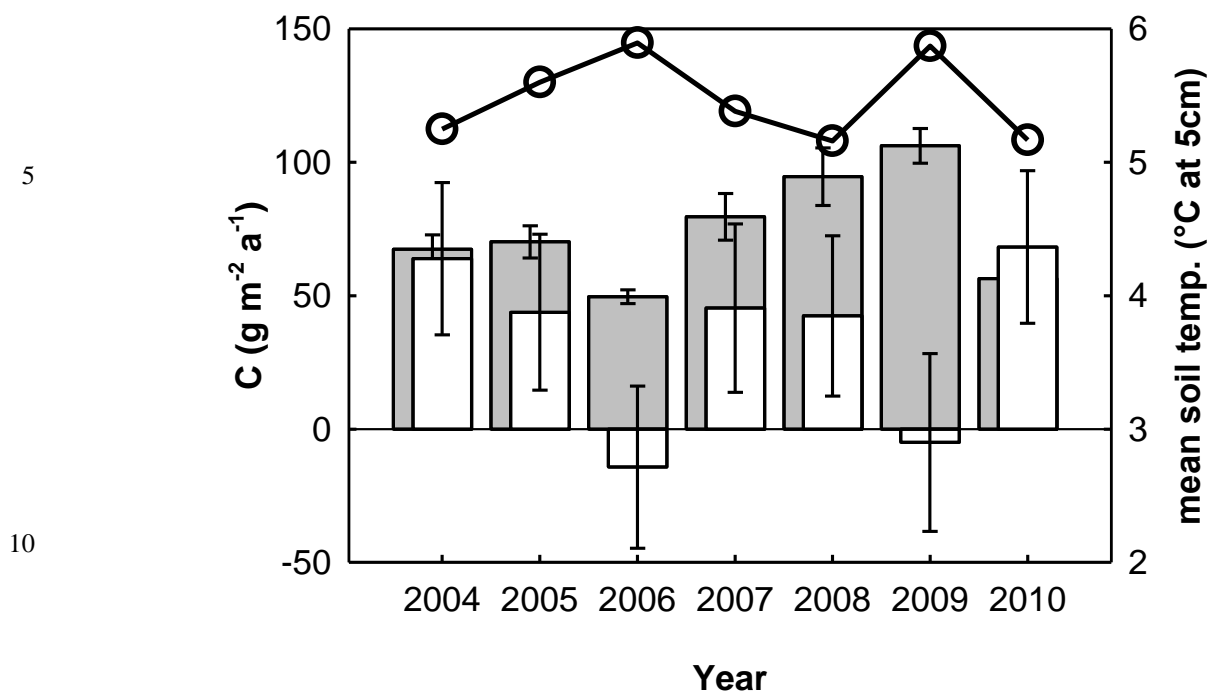
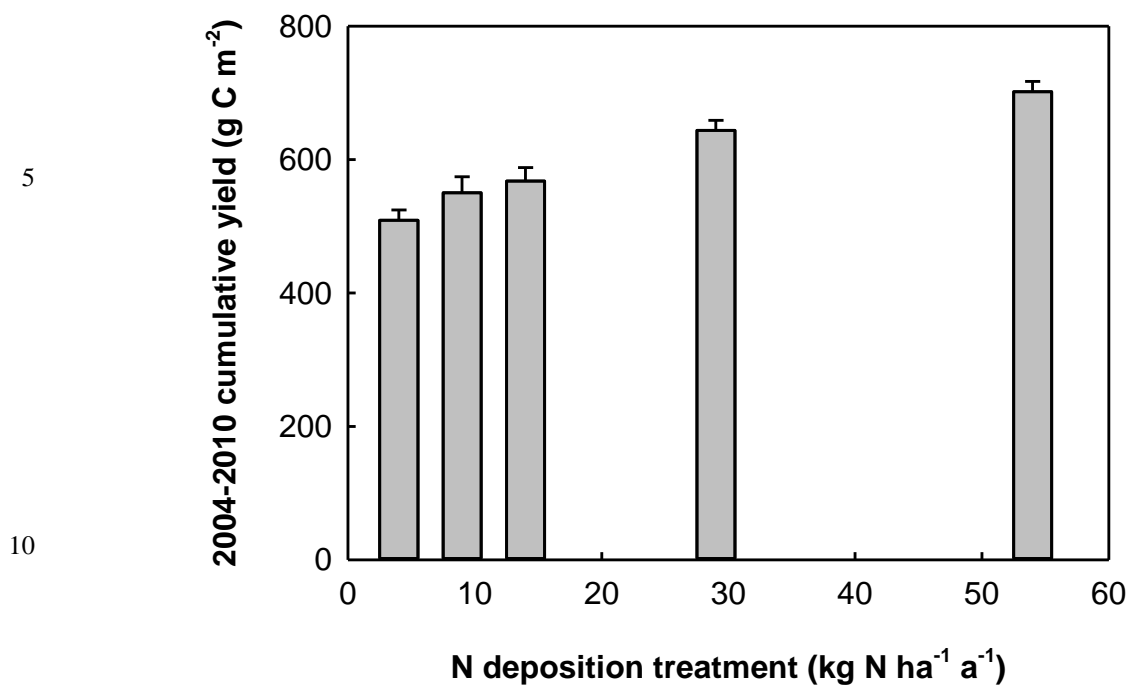



Figure 1 Aboveground plant yield (grey bars) and net ecosystem productivity (NEP; white bars) for the control treatment, and annual mean soil temperature at 5 cm depth (T_{soil}; solid line) for each year during the experiment. Soil temperature accuracy is ±0.18° C (within year systematic error for mean of nine probes used).

15 **Figure 2** Seven year cumulative aboveground plant C yield (O$_3$ treatments lumped), plotted against total annual N

deposition of 4, 9, 14, 27 and 54 kg N ha^{-1} yr^{-1} (atmospheric background plus experimental deposition rates).

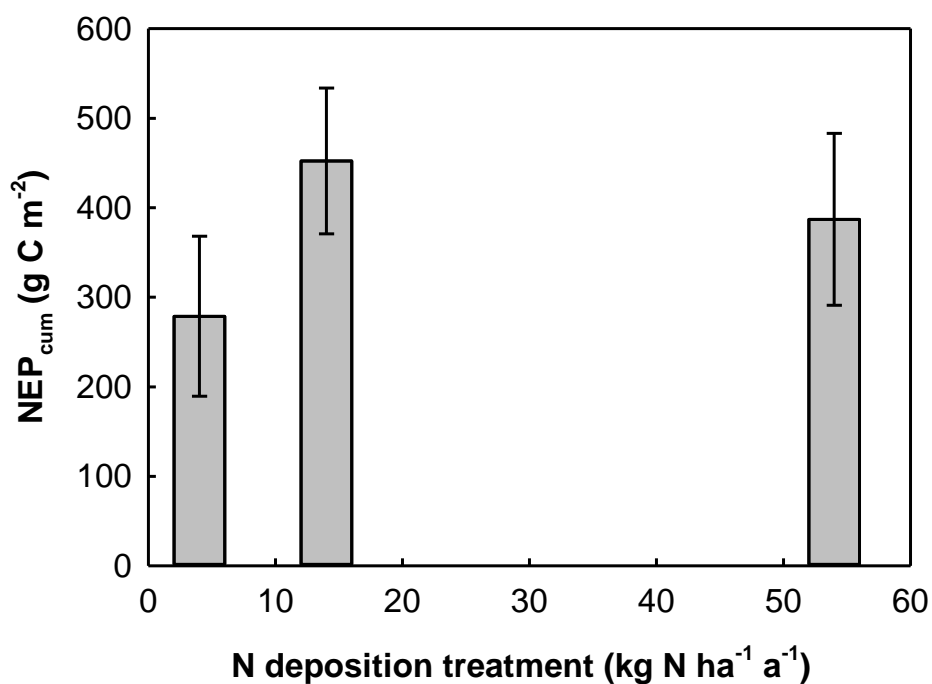



Figure 3 Seven year cumulative NEP carbon gain (O_3 treatments lumped), plotted against total annual N deposition of 4, 14 and 54 kg N ha^{-1} yr^{-1} (atmospheric background plus experimental deposition rates).

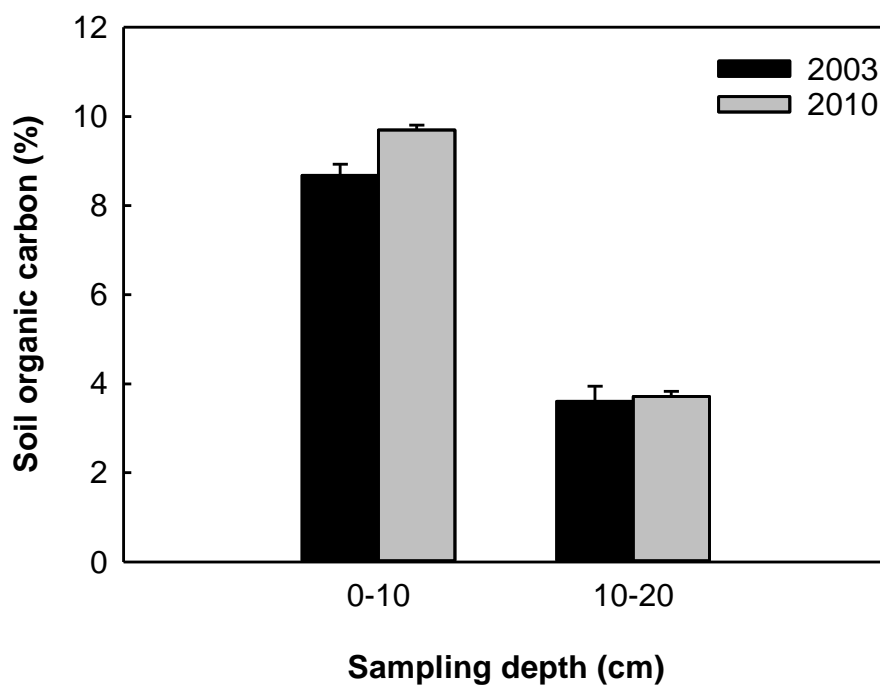



Figure 4 Bulk soil (control treatment) organic carbon concentration in 2003 (grey bars) and 2010 (black bars) in the 0-10 cm and 10-20 cm soil layer.

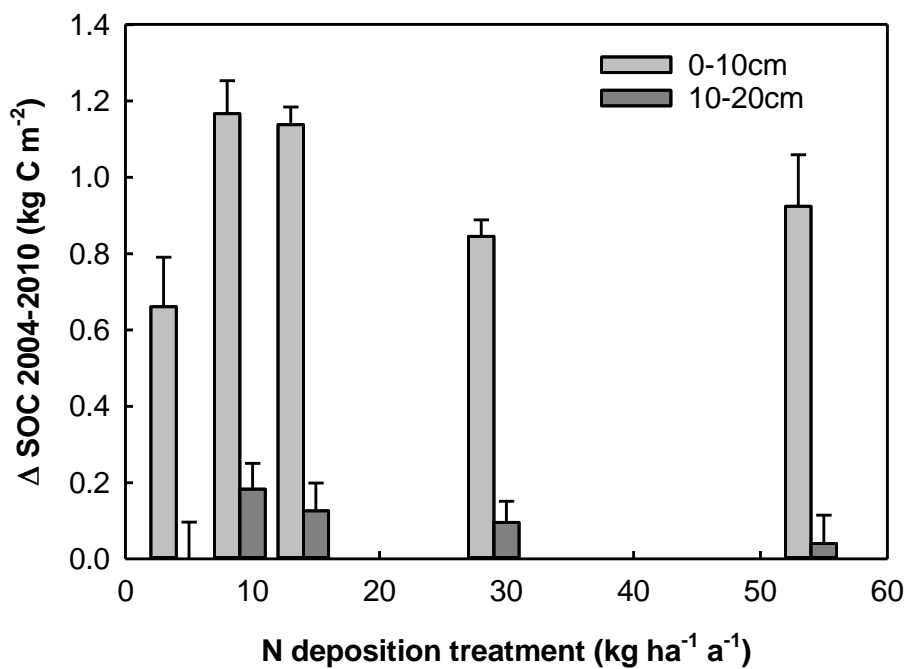



Figure 5 Absolute soil organic C stock gains during seven years (2003-2010) in the 0-10 cm (grey bars) and the 10-20 cm layer (black bars). O_3 treatments are lumped in N-treatment levels of 4, 9, 14, 27 and 54 kg N ha^{-1} yr^{-1} (atmospheric background plus experimental deposition rates).