

Nitrogen deposition but not ozone affects productivity and community composition of subalpine grassland after 3 yr of treatment

Seraina Bassin¹, Matthias Volk¹, Matthias Suter², Nina Buchmann³ and Jürg Fuhrer¹

¹Air Pollution and Climate Group, Agroscope Research Station ART, CH-8046 Zurich, Switzerland; ²Institute of Integrative Biology, ETH Zurich, CH-8092 Zurich, Switzerland; ³Institute of Plant Sciences, ETH Zurich, CH-8092 Zurich, Switzerland

Summary

Author for correspondence:

Seraina Bassin

Tel: +41 44 377 75 08

Fax: +41 44 377 72 01

Email: Seraina.Bassin@art.admin.ch

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- A field experiment was established at 2000 m above sea level (asl) in the central Swiss Alps with the aim of investigating the effects of elevated ozone (O₃) and nitrogen deposition (N), and of their combination, on above-ground productivity and species composition of subalpine grassland.
- One hundred and eighty monoliths were extracted from a species-rich *Geo-Montani-Nardetum* pasture and exposed in a free-air O₃-fumigation system to one of three concentrations of O₃ (ambient, 1.2 × ambient, 1.6 × ambient) and five concentrations of additional N. Above-ground biomass, proportion of functional groups and normalized difference vegetation index (NDVI) were measured annually.
- After 3 yr of treatment, the vegetation responded to the N input with an increase in above-ground productivity and altered species composition, but without changes resulting from elevated O₃. N input > 10 kg N ha⁻¹ yr⁻¹ was sufficient to affect the composition of functional groups, with sedges benefiting over-proportionally. No interaction of O₃ × N was observed, except for NDVI; positive effects of N addition on canopy greenness were counteracted by accelerated leaf senescence in the highest O₃ treatment.
- The results suggest that effects of elevated O₃ on the productivity and floristic composition of subalpine grassland may develop slowly, regardless of the sensitive response to increasing N.

Key words: *Carex*, grassland, *Nardetum*, nitrogen, ozone, seminatural, subalpine zone.

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Introduction

During the last century, concentrations in the troposphere of ozone (O₃) and reactive nitrogen compounds (N) have risen substantially on a global scale as a result of increasing anthropogenic emissions (Matson *et al.*, 2002; Ashmore, 2005). Today many regions are exposed to O₃ concentrations and N deposition rates that exceed preindustrial values several-fold (Volz & Kley, 1988; Preunkert *et al.*, 2003). In Switzerland, current annual rates of N deposition in remote

mountain areas are around 5 kg N ha⁻¹ yr⁻¹, but reach up to 60 kg N ha⁻¹ yr⁻¹ in regions with intensive livestock farming (Rihm & Kurz, 2001). Both N input and O₃ have been found separately to affect plant communities of natural and seminatural habitats in ways that threaten the structure and function of these systems (Ashmore, 2005; Phoenix *et al.*, 2006). To protect sensitive communities from excess pollutant input, effect-based abatement strategies applying critical concentrations for O₃ and critical loads for N have been developed under the UNECE Convention on Long-range Transboundary

Air Pollution (CLRTAP). However, owing to the lack of suitable experimental data, possible interactive effects of O₃ and N have not been considered.

As an essential nutrient, atmospheric N can alter ecosystem properties such as productivity, cycling of carbon (C) and N (Vitousek *et al.*, 1997) or biodiversity (Stevens *et al.*, 2004). In particular, in natural and seminatural ecosystems species characteristic of nutrient-poor conditions may be displaced by a few fast-growing and highly competitive species (Bobbink *et al.*, 1998). When, as a result of increasing nutrient availability, below-ground competition for resources shifts to above-ground competition for light, species that effectively invest available N into high relative growth rates are favoured (Schieving & Poorter, 1999), as observed in several N addition experiments in dune vegetation (van den Berg *et al.*, 2005) and calcareous grasslands (Bobbink, 1991). Herbaceous systems in climatically extreme environments such as mountain regions may be most susceptible to even low rates of N addition, as suggested by Bowman & Steltzer (1998). These systems are currently marginally exposed to air pollutants, and in the absence of high concentrations of N input they are typically nutrient-poor and species-rich, and thus of high conservation value. In the alpine zones, N addition experiments concentrated on unmanaged alpine heaths (Soudzilovskaia & Onipchenko, 2005) and tundra (Theodose & Bowman, 1997; Bowman *et al.*, 2006), but little is known about effects of increasing N inputs on subalpine grasslands which play an important role for both conservation and agriculture.

Ozone is the major phytotoxic air pollutant on regional scales. There is ample evidence that current ambient concentrations are sufficiently high to cause widespread visible O₃ injury in plants in Europe and elsewhere (Benton *et al.*, 2000). Elevated O₃ impairs photosynthesis, leaf life span, reproduction and growth (Ojanperä *et al.*, 1998). In species-poor productive grass/legume swards, experimental exposure to elevated O₃ consistently caused an increase in the dominance of grasses at the expense of legumes (cf. Fuhrer *et al.*, 1994). In contrast, in natural multispecies communities, few available studies revealed less consistent responses with either declining (Volk *et al.*, 2006) or rising proportions of legumes (Evans & Ashmore, 1992), and unaffected (Barbo *et al.*, 1998) or reduced total yield (Evans & Ashmore, 1992; Volk *et al.*, 2006). These conflicting results suggest that in complex mature communities, multiple plant–plant and plant–environment interactions might strongly modulate the effect of O₃.

In grasslands, large variability in responsiveness to O₃ stress exists among and within species (Bungener *et al.*, 1999; Bassin *et al.*, 2004b). Recently, the responses to O₃ of different species and community types have been compared (Bassin *et al.*, 2006; Jones *et al.*, 2006; Mills *et al.*, 2006). Bassin *et al.* (2006) discussed the importance of physiological and ecological traits in determining O₃ sensitivity. They suggested that a high relative growth rate with related leaf

traits such as high stomatal conductance and high specific leaf area (SLA) promote the specific sensitivity to O₃. Accordingly, low-productivity communities such as alpine and subalpine grasslands could be considered less responsive to O₃ because of their high proportion of slow-growing, stress-tolerant species. In contrast, Mills *et al.* (2006) classified this type of grassland among the most sensitive vegetation types based on individual species' responses to O₃ observed under controlled conditions.

Theoretically, an increase in N availability and in O₃ exposure could have opposing effects. Owing to phenotypic plasticity, plants might become more susceptible to O₃ when growth rates are stimulated by extra available N, or alternatively enhanced detoxification capacity from N-stimulated photosynthesis could reduce O₃ sensitivity. For instance, high nutrient supply protected *Plantago major* against O₃ damage (Whitfield *et al.*, 1998) and *Trifolium repens* from accelerated leaf senescence (Sanz *et al.*, 2005). However, data from *in situ* experiments with intact communities, including multiple treatment levels and factor combinations to test the interaction between N and O₃, are lacking.

This study was designed to investigate the long-term impact of elevated O₃ and N input, and of their combination, in extensively managed grassland in the Swiss Alps. The aim was to determine: (i) total above-ground productivity; (ii) productivity and proportion of functional groups (grasses, forbs, sedges and legumes); (iii) individual species' abundance and diversity; and (iv) the development of the vegetation greenness as an indicator of leaf senescence, in relation to individual and combined concentrations of O₃ and N deposition. Here we report results from the initial 3 yr of experiments.

Materials and Methods

Study area

The experimental site is located at Alp Flix, Sur, Switzerland (2000 m asl, 9°39'N/46°32'E), a high plateau near Julier Pass in the central Alps. The plateau, located *c.* 100 m below the climatic tree line, is dominated by a raised bog surrounded by extensively managed pastures and hay meadows. Climatic conditions are characterized by cold winters with permanent snow cover and a growing season from April to October. Precipitation peaks in summer (long-term average 120–140 mm monthly from May to September) with an annual sum of 1200 mm. Long-term mean annual temperature is 2.8°C, and monthly average temperatures are highest in July and August (9–10°C).

Vegetation and soil

The vegetation is a *Geo-Montani-Nardetum*, which is a typical pasture community covering large areas in the subalpine zone of the European Alps and Pyrenees (EUNIS classification

35.1, <http://eunis.eea.europa.eu/>). The most frequent species are *Festuca violacea*, *Nardus stricta* and *Carex sempervirens*, which together account for approximately one-half of the cover. The remaining half is composed of > 70 forb and a few legume species. The low-intensity management, as a cattle pasture with *c.* 1.3 livestock units ha^{-1} grazing during 3–4 wk yr^{-1} , was replaced in the present study by a cutting regime. It is uncertain to what extent above-ground productivity and species composition might be affected in the long term as a result of the release from grazing selection and potential modifications of the nutrient turnover.

The soil is a slightly acidic cambisol (pH 4.8–6) developed on Serpentine bedrock. Soil depth varies between 20 and 40 cm, but rooting depth is limited to a maximum of 20 cm.

Experimental design, fumigation and N deposition treatment

A free air fumigation system was installed in spring 2004, consisting of nine rings of 7 m diameter (for details of the system and of its performance, see Volk *et al.*, 2003). Rings were arranged in three linear blocks on a small ridge of length 150 m. The ridge is orientated east–west and orthogonal to the two main wind directions. Three fumigation treatments (ambient (control), $1.2 \times$ ambient (O_3+), $1.6 \times$ O_3 ambient concentration (O_3++)) were randomly assigned to the three rings in each block. With O_3 concentrations at these altitudes being mainly controlled by the global background concentration (Staehelin *et al.*, 1994), we aimed to increase O_3 concentration during the day as well as during the night. Exposure to elevated O_3 concentrations lasted from 16 June to 25 October in 2004, from 15 April to 24 October in 2005, and from 21 April to 23 October 2006. In 2004, late snow-melt delayed the installation of the fumigation system. Meteorological conditions allowed O_3 release 74, 62 and 78% of the time during the fumigation periods in the years 2004, 2005, and 2006, respectively. O_3 concentrations were measured at canopy height in control and fumigation treatments and at reference height (2 m) in ambient air.

Experimental plots consisted of monoliths ($L \times W \times D = 40 \times 30 \times 20$ cm). These were excavated in autumn 2003 from a nearby pasture, placed in drained plastic boxes and randomly assigned to the rings. Boxes were placed in shallow pits flush with the surrounding surface. They were rearranged every year between the rings within each O_3 treatment to minimize confounding effects of microclimatic differences between rings.

Five concentrations of N application were established to simulate atmospheric N deposition during the growing season. Extra N input in these treatments was equivalent to 0, 5, 10, 25, 50 $\text{kg N ha}^{-1} \text{yr}^{-1}$. These annual N amounts were separated into biweekly additions of 200 ml of a solution of ammonium nitrate in well water. Each N concentration was replicated four times in each fumigation ring. The amount of added water was equivalent to only 2% of the annual rainfall

amount, and N addition from well water was $< 0.05 \text{ kg N ha}^{-1} \text{yr}^{-1}$. Considering the annual background deposition measured at the study site (*c.* 4 $\text{kg N ha}^{-1} \text{yr}^{-1}$, see the Results section), total N loads in the different treatments were 4, 9, 14, 29 and 54 $\text{kg N ha}^{-1} \text{yr}^{-1}$. In the following, treatments are referred to as N4, N9, N14, N29 and N54, respectively.

Micrometeorological measurements

Meteorological parameters were recorded at 2 m reference height outside the fumigation rings: global radiation (W m^{-2}), wind speed (m s^{-1}), air temperature ($^{\circ}\text{C}$), relative humidity (%), and precipitation (mm h^{-1}). For details concerning measurement equipment see Volk *et al.* (2003).

Background N deposition measurements

Background N deposition was calculated from concentrations measured in air, rainwater and snow. During the growing season, biweekly average ammonia (NH_3) and nitrogen dioxide (NO_2) concentrations in air were determined by means of passive samplers (NH_3 , Radiello® Fondazione Salvatore Maugeri, Pavia, Italy; NO_2 , constructed after Palmes *et al.*, 1976)). Fluxes of $\text{NH}_3\text{-N}$ and $\text{NO}_3\text{-N}$ were estimated as a function of the serial resistances R_a (aerodynamic resistance), R_b (boundary layer resistance), R_s (stomatal resistance), and, in the case of NH_3 , R_w (leaf surface resistance), following the parameterization proposed by Bassin *et al.* (2004a) and Smith *et al.* (2000). Total gaseous N deposition per growing season (1 May–31 October) was calculated as the sum of hourly fluxes. Monthly rates of wet deposition of ammonium (NH_4^+) and nitrate (NO_3^-) were obtained from concentrations measured in rainwater collected in a wet-only sampler (Aerochem Metrics Inc., Bushnell, FL, USA). During winter, NH_4^+ and NO_3^- loads of the snow pack were measured shortly before snow-melt, assuming that at this time the snow pack contained the accumulated wet and dry deposition.

Chemical analysis of NO_2 in passive samplers (as nitrite), and NH_4^+ in rainwater and melted snow were measured by spectrophotometry (Lambda 25, Perkin Elmer, Shelton, CT, USA). NH_4^+ from NH_3 in passive samplers and NO_3^- in water were analysed by ionchromatography (DX1-03, DIONEX, Sunnyvale, CA, USA). In 2006, the methods for the determination of NH_4^+ were replaced by flow injection analysis (Fiastar, FOSS, Hillerød, Denmark).

Vegetation measurements

Plants were cut at 2 cm above the surface once each year in August. Plant material was stored at 4°C until separation into functional groups (grasses, forbs, sedges, and legumes). Samples were dried at 60°C to constant weight.

The frequency of individual plant species was recorded each year during the first 2 wk of July using the point-quadrat

(PQ) method (Stampfli, 1991) with 18 sampling points within a 6 cm grid. Based on these values, canopy density (= total number of PQ hits per monolith) as well as diversity index were derived (Shannon-Wiener index, H') according to the following:

$$H' = \sum_{i=1}^s p_i \ln p_i \quad \text{Eqn 1}$$

(s , species number; p_i , relative abundance of the i th species).

Vegetation development was monitored by measuring the normalized difference vegetation index (NDVI) at 2-wk intervals during June and July. In sparse canopies, NDVI provides an integrative measure of leaf area index (LAI) and chlorophyll content of the vegetation (Gamon *et al.*, 1995). A multispectral radiometer sensor (MSRSYS16R system, Cropscan Inc., Rochester, MN, USA) to measure reflectance at 810 and 680 nm was placed 90 cm above the centre of a monolith. During the measurement, the surrounding monoliths were covered. Measurements were taken only on cloudless days during hours with a sun angle $> 60^\circ$ (from 10 am to 5 pm). NDVI was calculated following Tucker (1979):

$$\text{NDVI} = (\text{NIR} - \text{RED}) / (\text{NIR} + \text{RED}) \quad \text{Eqn 2}$$

(RED and NIR, the measured spectral reflectance in the red and near-infrared regions, respectively).

Data analysis

Effects of treatments on above-ground dry matter weight, proportions of functional groups, species number per monolith, diversity index H' , and NDVI were tested in a repeated-measures split-plot analysis of variance (ANOVA) with O_3 at the main-plot level and N at the subplot level. All parameters except NDVI were repeated by year; NDVI was analysed separately for the two years 2005 and 2006 and repeated by day of the year. Block, O_3 , and year entered the model as class variables, while N and day (NDVI) were defined as continuous variables. Inclusion of higher-order interactions and quadratic effects into the model were assessed with the AICc criterion (Burnham & Anderson, 2002) with one exception: the interaction of $\text{N} \times \text{N}$ was always included since it refers to the curvature of N as a design variable. To evaluate differences between dry matter weight of functional groups at different N concentrations, values were grouped by N concentration separately for 2005 and 2006, and a one-way ANOVA followed by a Tukey post-hoc test was performed. Treatment-induced changes in the abundance of the 11 most frequent species were analysed qualitatively. All analyses were carried out with the statistical software SAS (SAS Institute Inc., Cary, NC, USA), applying the procedures 'proc mixed' and 'proc anova'. To meet the assumptions of ANOVA (normal distribution and homogeneity of residual variance), data were

transformed when necessary. To account for the multidimensional data structure appropriately, estimated means and standard errors (SE) resulting from back-transformation are displayed in figures and the text.

Results

Climatic conditions, O_3 exposure, background N deposition

Weather conditions varied considerably over the 3 yr (Table 1) with the summer of 2004 being the wettest, and that of 2006 the warmest and driest. Overall, the region experienced warmer and drier years in 2004–06 compared with the long-term annual mean (data from Swiss Meteorological Service, <http://www.meteosuisse.ch/web/en/weather.html>).

Ambient O_3 concentrations (24 h means) during the fumigation period were *c.* 46 ppb (Table 1), showing only a small diurnal variation. Accumulated exposures above a threshold of 40 ppb (AOT40) averaged 12 ppm h in the control plots, 24 ppm h in the O_3+ treatment and 48 ppm h in the O_3++ treatment. Year-to-year variations in AOT40 in the elevated O_3 treatments were associated with variations in meteorological conditions and variable length of exposure duration.

Estimated total annual N deposition was between 3.2 and 3.8 kg N ha⁻¹ yr⁻¹, with 1.33–2.7 kg N ha⁻¹ yr⁻¹ deposited with rain and *c.* 0.5 kg N ha⁻¹ yr⁻¹ as dry deposition (Table 1). Winter N input to the vegetation was low (0.14–0.58 kg N ha⁻¹ yr⁻¹) because of the small amount of snow accumulation.

Total above-ground biomass

Above-ground productivity generally did not respond to elevated O_3 during the 3 yr (Fig. 1, Table 2). The O_3 effect pattern was not consistent over time, as indicated by the significant $\text{O}_3 \times \text{year}$ interaction: in year 2004, there was a reduction of total dry matter with O_3+ (Fig. 1). N addition persistently stimulated biomass, but no $\text{O}_3 \times \text{N}$ interaction was observed (Fig. 1a, Table 2). Growth stimulation in the N29 treatment relative to control plots was +24% ($P < 0.05$, Tukey test for multiple comparisons) in 2005 and +25% ($P < 0.05$) in 2006 (Supplementary material, Fig. S1), while in the N54 treatment, the growth increase amounted to +34% in both years ($P < 0.05$ in both years). In the N9 treatment, biomass was also slightly, but nonsignificantly, stimulated by 8 and 6% in the 2 yr, respectively, and in the N14 treatment by 13 and 10%, respectively.

Harvested above-ground biomass differed significantly among the years (Table 2). In control plots, it dropped from *c.* 100 g m⁻² in 2004 and 2005–83 g m⁻² in 2006 (Fig. 1a). So far it is unclear whether this drop was caused by the dry weather conditions in 2006 (Table 1) or whether it is an early indication of long-term effects of the management change.

Table 1 Mean temperatures, sum of precipitation, wind velocity and AOT40, mean ozone (O_3) concentrations, and mean daily O_3 concentration maxima measured in the O_3 fumigation experiment at Alp Flix during the growing seasons (April–October) in 2004–06, as well as the contribution of reduced (NH_3 , NH_4^+) and oxidized (NO_2 , NO_3^-) N to the annual total N deposition, according to deposition form (winter, snow pack; summer, wet and dry deposition)

	2004			2005			2006		
	Temperature (°C)	Precipitation (mm)	Wind speed (m s ⁻¹)	Temperature (°C)	Precipitation (mm)	Wind speed (m s ⁻¹)	Temperature (°C)	Precipitation (mm)	Wind speed (m s ⁻¹)
	5.6	722.6	3.3	7.2	488.7	3.2	7.0	368.7	3.6
	AOT40 (ppm h)	Mean conc. (ppb)	Daily max. (ppb)	AOT40 (ppm h)	Mean conc. (ppb)	Daily max. (ppb)	AOT40 (ppm h)	Mean conc. (ppb)	Daily max. (ppb)
Ambient	13.8*	46.9*	55.9*	15.4	45.7	52.8	14.9	44.9	53.1
Control	12.6**	46.0**	54.7**	11.1	44.0	50.8	11.6	42.1	50.3
O_3 +	15.2	51.2	61.0	27.4	51.6	64.5	29.5	54.9	70.0
O_3 ++	28.4	64.2	81.5	49.9	65.0	84.7	64.9	74.2	101.7
	Snow pack (kg N ha ⁻¹ wi. ⁻¹)	Wet deposition (kg N ha ⁻¹ su. ⁻¹)	Dry deposition (kg N ha ⁻¹ su. ⁻¹)	Snow pack (kg N ha ⁻¹ wi. ⁻¹)	Wet deposition (kg N ha ⁻¹ su. ⁻¹)	Dry deposition (kg N ha ⁻¹ su. ⁻¹)	Snow pack (kg N ha ⁻¹ wi. ⁻¹)	Wet deposition (kg N ha ⁻¹ su. ⁻¹)	Dry deposition (kg N ha ⁻¹ su. ⁻¹)
NH_4^+ -N	na	0.75	0.53	0.23	1.67	0.45	0.05	1.53	0.64
NO_3^- -N	na	0.58	0.05	0.35	1.05	0.06	0.09	0.82	0.06

na, not available; wi., winter; su., summer.

*, Reconstructed for the entire growth period with values from the O_3 -monitoring station at Davos (1590 m asl).

**, Reconstructed for the period of 16 August to 20 October with values from the O_3 -monitoring station at Davos (1590 m asl).

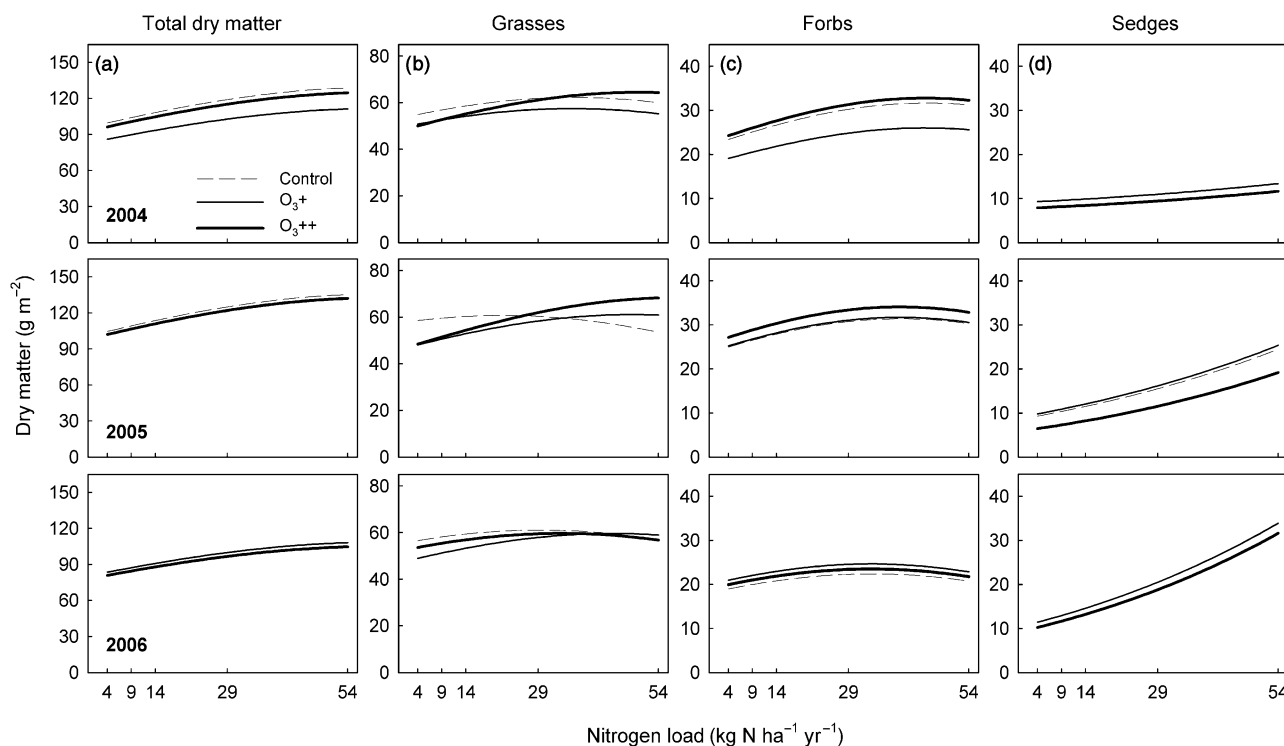


Fig. 1 Total above-ground total dry matter (a) and dry matter of the functional groups of grasses (b), forbs (c) and sedges (d) as a function of ozone (O_3), nitrogen deposition (N), and year. To allow a clear interpretation, only fitted lines based on the ANOVA model (Table S2) are displayed.

Dry matter and proportion of functional groups

Nitrogen addition but not O_3 modified the functional group composition with no significant $O_3 \times N$ interaction. The group of sedges benefited most from the additional N input and the N-induced increase in biomass became more pronounced over the years (Fig. 1d). In 2006, even the N14 concentration (+55%) was significantly different from the N4 concentration ($P < 0.05$, Fig. S1b). Biomass of sedges was simulated by 124 and 257% in the N29 and N54 treatments, respectively ($P < 0.05$). In terms of proportions, the fraction of sedges increased from 11% in the control plots to 14% in the N54 treatment in 2004, and more than doubled from 14% (N4) to 31% (N54) in 2006 (Table 2: year \times N interaction); thus sedges became the second most abundant functional group in this treatment (Fig. 2).

Grasses as the dominating functional group with an average dry matter yield of around 60 g m^{-2} did not benefit from the extra N input (Table S2). As a result, their fraction declined in the N54 treatment progressively from 2004 to 2006 (Fig. 2, Table 2: year \times N interaction).

By contrast, forbs with approximately 30 g m^{-2} were significantly promoted by N addition, but the stimulating effect declined during the 3 yr (Fig. 1c), and in 2005 and 2006 differences between N treatments disappeared (Fig. S1). The fraction of forbs declined from 27% (2005 and 2006) in the

N4 treatment to 24% (2005) and to 22% (2006) in the N54 treatment (Fig. 2, Table 2: year \times N interaction).

Dry matter of legumes ranged between 2 and 3 g m^{-2} and accounted for only 2–5% of the total biomass (Fig. 2). Statistical analyses were not feasible because of the heterogeneous distribution among plots, but N addition tended to slightly reduce the fraction of legumes, while neither O_3 nor the $O_3 \times N$ interaction caused a detectable response.

Species composition and frequency

The total number of vascular species was 91 (Table S1) with 10% grasses, 7% sedges, 4% legumes, and a majority (79%) of forbs. The dominating species with an average of more than five point-quadrat hits (PQ hits) were the grasses *F. violacea* and *N. stricta*, and the sedge *C. sempervirens* (Table 3). The six most frequent forb species (*Ranunculus villarsii*, *Leontodon helveticus*, *Ligusticum mutellina*, *Potentilla aurea*, *Arnica montana* and *Gentiana acaulis*), and one legume species (*Trifolium alpinum*) averaged between 0.5 and 1.7 PQ hits, while the majority (80 species) touched the probe on the average < 0.5 times per monolith (0.12 m^2). Neither the number of species per monolith nor the Shannon diversity index was influenced by O_3 or N treatments, but the total species number differed significantly among years (Table 2).

Table 2 Effects of ozone (O_3), nitrogen deposition (N), and year on total harvested above-ground biomass, the proportion of grasses, forbs and sedges, and on the total number of point-quadrat hits (PQ hits), species number, and Shannon diversity index H' in monoliths of a free-air fumigation experiment at Alp Flix in 2004–06

	Total dry matter			Grasses proportion			Forbs proportion			Sedges proportion			No. of PQ hits			Species No.			Shannon index H'		
	N d.f.	D d.f.	F	N d.f.	D d.f.	F	N d.f.	D d.f.	F	N d.f.	D d.f.	F	N d.f.	D d.f.	F	N d.f.	D d.f.	F	N d.f.	D d.f.	F
Block	2	4	5.83	2	4	1.30	2	4	4.41	2	4	0.85	2	4	4.60	2	4	6.62	2	4	0.25
O_3	2	4	0.56	2	4	0.52	2	4	0.67	2	4	1.25	2	4	1.17	2	4	0.73	2	4	0.92
N	1	169	28.99***	1	169	9.20**	1	169	1.11	1	169	19.50***	1	169	23.78***	1	169	3.12	1	169	0.28
N × N	1	169	1.46	1	169	0.29	1	169	0.84	1	169	0.26	1	169	1.37	1	169	0.09	1	169	0.10
Year	2	350	65.47***	2	356	62.05***	2	352	3.35*	2	352	190.96***	2	350	110.87***	2	350	13.22***	2	358	2.11
Year × block	4	350	6.26***	—	—	—	4	352	4.33**	—	—	—	4	350	6.39***	4	350	5.15***	—	—	—
Year × O_3	4	350	3.44**	—	—	—	—	—	—	4	352	4.24**	4	350	6.22***	4	350	3.05*	—	—	—
Year × N	—	—	—	2	356	8.03***	2	352	12.19***	2	352	38.39***	—	—	—	—	—	—	—	—	—

Numerator (N d.f.) and denominator degrees of freedom (D d.f.) and F -values are displayed. Terms with '—' were not fitted in the particular model. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Corresponding to measured dry matter, N but not O_3 affected the abundance of the most frequent 11 species (Table 3). In some cases, individual species reflected the performance of the functional group to which they belong. For instance, *C. sempervirens* as the most abundant sedge showed an N response similar to the respective functional group. Among the grasses, only the fraction of *N. stricta* decreased in response to N addition (Table 3), while *F. violacea* and *Helictotrichon versicolor* remained unaffected. In the group of forbs, N affected *P. aurea* positively and *A. montana* negatively, while all other forbs did not respond to any of the treatments. The legume *T. alpinum* declined with increasing N addition.

NDVI

Normalized difference vegetation index clearly reflected differences in phenological development. NDVI peaked on 10 July 2005 at values between 0.82 and 0.85, followed by a slight decline owing to the senescing vegetation (Fig. 3a). In 2006, NDVI only reached values between 0.72 and 0.75 without a detectable maximum, thus indicating that the vegetation developed only marginally after the start of the measurements (Fig. 3b). This pattern corresponded to the comparatively low dry matter yield in 2006 (Fig. 1a).

In both years, NDVI increased with higher N concentrations (Fig. 3, Table 4). In the O_3++ treatment, this effect was less pronounced (nonsignificant trend in 2005) or marginal (2006, Table 4: $N \times O_3$ interaction). In both years, NDVI curves levelled off only in the O_3++ treatment, which resulted in a significant day $\times O_3$ interaction (Table 4).

Discussion

Elevated N deposition

The measured background N deposition of 3–4 kg N ha⁻¹ yr⁻¹ corresponds to deposition rates measured at comparable alpine sites in the central Swiss Alps (Hiltbrunner *et al.*, 2005). Such alpine and subalpine ecosystems together with moorland and heathland habitats in northern Europe (Smith *et al.*, 2000; Fremstad *et al.*, 2005) represent relic sites with little influence from anthropogenic N input in the past. In most of the published studies on herbaceous plant communities, the simulated N deposition rates by far exceeded the range of anthropogenic deposition for the respective study site (Theodose & Bowman, 1997; Soudzilovskaia & Onipchenko, 2005). However, such efforts to shortcut long-term accumulation of low N deposition rates disregard the boosting effects of an abrupt rise in N availability on soil microorganisms and plants. Therefore, long-term experiments, including high-resolution treatments between 5 and 50 kg ha⁻¹ yr⁻¹ in regions with low background N deposition, are required to elucidate the effects of chronic low-level N deposition on nutrient poor vegetation (Achermann & Bobbink, 2003).

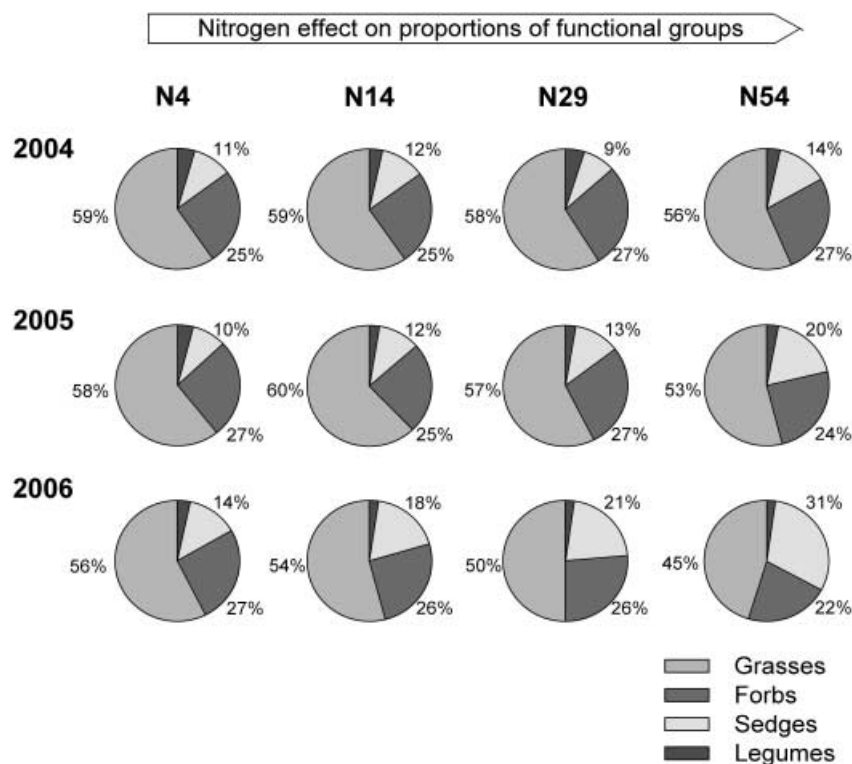


Fig. 2 Relative contribution to the total harvested above-ground biomass of the four functional groups (grasses, forbs, sedges and legumes) in relation to the nitrogen deposition (N) treatments and the three experimental years (2004–06).

	Functional group	Frequency (no. of PQ hits)	Effect of O ₃	Effect of N
<i>Festuca violacea</i>	Grasses	7.7 ± 1.1	No	No
<i>Carex sempervirens</i>	Sedges	5.9 ± 0.5	No	++
<i>Nardus stricta</i>	Grasses	5.0 ± 0.7	No	–
<i>Ranunculus villarsii</i>	Forbs	1.7 ± 0.3	No	No
<i>Trifolium alpinum</i>	Legumes	1.5 ± 0.2	No	–
<i>Leontodon helveticus</i>	Forbs	1.4 ± 0.1	No	No
<i>Ligusticum mutellina</i>	Forbs	1.4 ± 0.1	No	No
<i>Potentilla aurea</i>	Forbs	1.3 ± 0.3	No	+
<i>Helictotrichon versicolor</i>	Grasses	1.2 ± 0.3	No	No
<i>Arnica montana</i>	Forbs	0.8 ± 0.1	No	–
<i>Gentiana acaulis</i>	Forbs	0.6 ± 0.1	No	No

Table 3 The 11 most abundant species observed in the ozone (O₃) fumigation experiment at Alp Flix, their functional group, the 3 yr average frequency per monolith in the control plots (no. of point-quadrat hits ± SE), and the extent or direction of their response to the O₃ and nitrogen deposition (N) treatments, respectively

Increased N deposition caused quick and strong changes in dry matter production and species composition. Owing to the lack of suitable experiments in the subalpine zone, these results can only be compared with those of studies in the alpine zone. However, alpine and subalpine grasslands have similar edaphic and climatic conditions and share various component species and characteristics, such as sparse canopies, prevailing slow-growing plants of low stature, or small and often needle-like leaves. In different studies, alpine vegetation did not respond in a consistent way to N addition, with stimulated productivity in some cases (Theodose & Bowman, 1997; Soudzilovskaia & Onipchenko, 2005) and no change in others (Bowman *et al.*, 2006). The discrepancies might be

related to differences in N-binding capacity of alpine soils (Körner, 2003), or to a colimitation by other macronutrients such as phosphorus (P) (e.g. in Theodose & Bowman, 1997; Soudzilovskaia & Onipchenko, 2005).

The N-induced change in vegetation composition was determined largely by an increase in sedges. Productivity of the other functional groups, species number per monolith, and evenness were not negatively affected by the success of sedges, in contrast to decreasing species richness generally found in lowland communities after N addition (Bobbink *et al.*, 1998). A similar increase in the cover of a few responsive species without a concomitant decrease in productivity of other species was observed in alpine plant communities

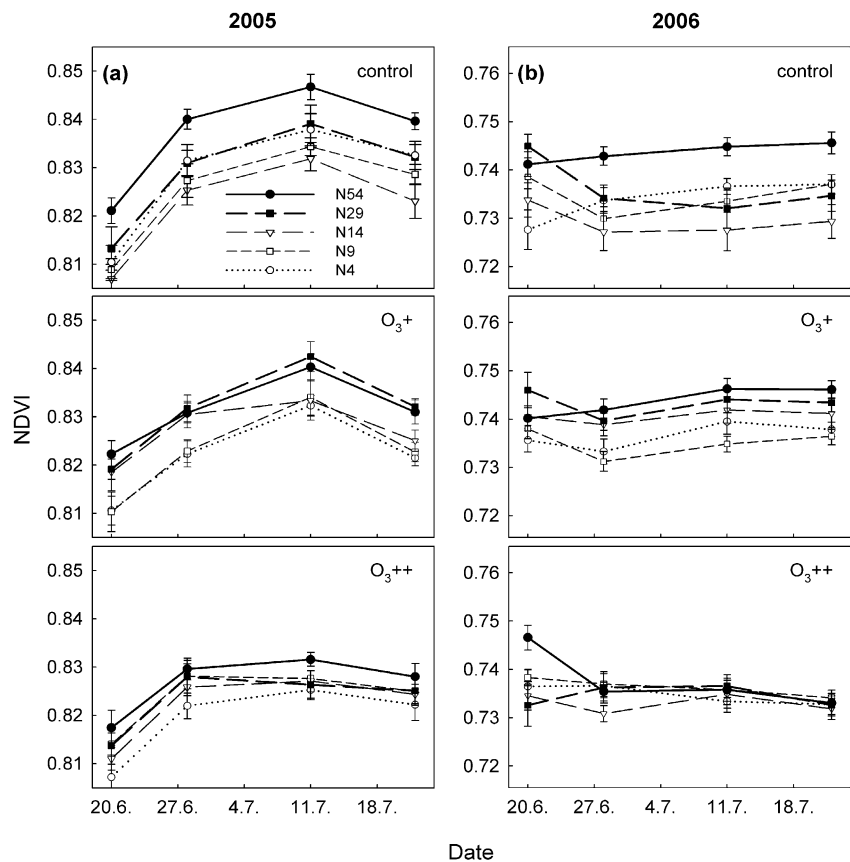


Fig. 3 Normalized difference vegetation index (NDVI, means \pm SE) during June and July of 2005 (a) and 2006 (b) in plots exposed to different concentrations and combinations of nitrogen deposition (N) and ozone (O_3).

Table 4 Effects of ozone (O_3), nitrogen deposition (N), and day on normalized difference vegetation index (NDVI) in vegetation monoliths of a free-air fumigation experiment at Alp Flix in 2005 and 2006

	NDVI 2005			NDVI 2006		
	N d.f.	D d.f.	F	N d.f.	D d.f.	F
Block	2	4	0.42	2	4	4.19*
O_3	2	4	4.18	2	4	8.72**
N	1	170	31.24***	1	168	25.87***
$N \times O_3$	–	–	–	2	168	3.46*
Day	1	551	382.97***	1	503	0.53
Day \times day	1	551	509.54***	–	–	–
Day $\times O_3$	2	551	13.02***	2	503	6.25**
Day \times day $\times O_3$	2	551	10.10***	–	–	–

Numerator (N d.f.) and denominator degrees of freedom (D d.f.) and *F*-values are displayed. Terms with '–' were not fitted in the particular model.

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

(Soudzilovskaia & Onipchenko, 2005; Bowman *et al.*, 2006). It was suggested that limited competition for light in sparse alpine canopies (Körner, 2003) allows unresponsive species to maintain their productivity.

Alpine studies consistently found sedges to benefit more from experimental N addition than grasses and forbs (Theodose & Bowman, 1997; Soudzilovskaia & Onipchenko, 2005).

This contradicts the common theory (e.g. C-S-R, Grime *et al.*, 1996) that species with a competitive growth strategy should benefit most. But according to Aerts (1999), in nutrient-limited habitats, typical sedge traits such as low nutrient loss through low tissue concentration, slow turnover rates and high resorption efficiency (Shane *et al.*, 2006) are more important for competitive success than investing into high growth rates.

Elevated O₃

After 3 yr of fumigation, elevated O₃ concentrations had no detectable effects on harvested above-ground dry matter and species composition of the subalpine grassland. The most likely explanation for the absence of a response to O₃ involves specific characteristics of this old, multispecies community. More pronounced O₃ effects were observed in past studies using individual plants, monocultures or binary mixtures of newly sown plants (Fuhrer *et al.*, 1994). Owing to their high growth rates, high stomatal conductance and high SLA, and in the absence of a large rooting system, plants in the establishing phase are assumed to be most sensitive to O₃, whereas established communities generally show weaker O₃ responses (Bassin *et al.*, 2006). Transient or absent O₃ effects on dry matter production were also observed in open-top chamber experiments with established vegetation (Evans & Ashmore, 1992; Thwaites *et al.*, 2006) and were explained by the large heterogeneity of the community composition as a result of edaphic and weather conditions. In a previous free-air fumigation experiment, productivity of old seminatural grassland was reduced only after 4 and 5 yr of exposure to elevated O₃ (Volk *et al.*, 2006). The old communities' ability to tolerate elevated O₃ concentrations has been related to functional redundancy, interspecific interactions, a large rooting system, and high genetic diversity (Bassin *et al.*, 2006).

Alternatively, the absence of a strong O₃ effect might be the result of the species composition of the monoliths. Low-productivity vegetation such as subalpine grassland is mainly composed of species with a stress-tolerant growth strategy, which have been considered unresponsive to O₃ (Bassin *et al.*, 2006). Adaptation to tolerate low nutrient availability and climatic conditions at the respective sites might protect (sub)alpine plant species from O₃ damage. In particular, the capacity to cope with O₃-induced oxidative stress could be pronounced in species from elevated sites because of their high amounts of antioxidants as an adaptation to natural oxidative stress by UV-radiation (Wildi & Lutz, 1996) and historically higher average O₃ concentrations (Staehelin *et al.*, 1994). Moreover, the monoliths contained only small proportions of legumes as their abundance decreases with elevation, and their altitudinal distribution is limited to below 2600 m asl (Jacot *et al.*, 2000). In a large number of studies, legumes consistently reacted highly sensitively to elevated O₃ concentrations (Hayes *et al.*, 2006), presumably because of their high relative growth rates and their specific chemical response to oxidative stress (Ranieri *et al.*, 2002). Accordingly, in grass/clover mixtures as well as in multispecies communities, observed changes in species composition resulted predominantly from a reduction in the legume fraction (Fuhrer *et al.*, 1994; Nussbaum *et al.*, 2000). In the present situation, an O₃-induced reduction in legumes would not lead to strong compositional changes because of their low abundance. Legume-poor communities could also be less sensitive to O₃ because

they depend less on N supplied by N fixation, which is often impaired by O₃ stress (Rämö *et al.*, 2006).

Lastly, the absence of O₃ effects could be related to the fumigation regime and/or the experimental design. The constant enrichment of background air during the entire growing season led to a seasonal O₃ exposure (AOT40) that was substantially higher than in previous studies (for comparison, see Rämö *et al.*, 2006; Thwaites *et al.*, 2006). Mean O₃ concentrations in ambient air persistently exceeded 40 ppb, and AOT40 of control plots exceeded by far the critical level for protecting seminatural vegetation of 5 ppm h over a 6 month period (CLRTAP, 2004). However, peak O₃ concentrations above 120 ppb that have been found to affect plants most effectively (Nussbaum *et al.*, 1995) were absent. Finally, the low number of replication at main plot level (O₃) limits the power of the experiment to detect significant O₃ effects.

Interactive effects of elevated O₃ and N deposition

After 3 yr, no interactive effect of O₃ and N deposition on productivity and species composition was detected, thus suggesting that possible modifications in leaf morphology and stomatal conductance associated with increased growth as a result of N addition did not affect the specific sensitivity to O₃. The alternative hypothesis that increased N availability would reduce O₃ sensitivity could not be tested because of the absence of an O₃ effect in the monoliths receiving no additional N.

An interactive effect of O₃ and N appeared for NDVI. N increased NDVI, but the effect was reduced in the O₃++ treatment. The N-induced increase in NDVI can be explained by a higher canopy density (PQ hits) and by increased chlorophyll content, as shown in Bell *et al.* (2004). Since there is no O₃ × N interaction in any measure of productivity, we can assume that the observed O₃ and O₃ × N effects on NDVI was rather the result of declining chlorophyll contents. O₃-induced reductions in NDVI in crops have been related to visible and nonvisible O₃ damage (Kraft *et al.*, 1996). The leaves of some of the species in the monoliths showed visible O₃ symptoms exclusively in the O₃++ treatment (e.g. *A. montana*), but more pronounced early leaf senescence was observed in the form of yellowing leaf tips (e.g. in *C. sempervirens*, *N. stricta*). Early leaf senescence is a common symptom of O₃ stress leading to reduced leaf longevity and C assimilation (Grandjean & Fuhrer, 1989). It could thus be expected that on longer timescales a continuous reduction of assimilation might affect the plants' C budget and, consequently, lead to long-term effects on productivity.

Conclusions

After 3 yr of treatment under realistic environmental conditions, it can be concluded that N inputs in the range of the current critical load for alpine and subalpine grassland in Europe of

10–15 kg N ha⁻¹ yr⁻¹ stimulate the productivity of a *Geo-Montani-Nardetum* pasture at 2000 m and alter community composition in favour of a few species, mainly sedges. When considering the possibility of N accumulation in the system, it seems likely that under conditions of low N export and nonlimiting P availability in soils, even lower concentrations of N deposition could induce vegetation changes in the long term. Such long-term effects merit further studies.

Productivity and community composition does not respond to 3 yr of increased O₃ exposure above current ambient concentrations. The lack of an O₃ response in spite of high cumulative exposures (AOT40) suggests considerable O₃ tolerance of the system. Inherent community characteristics or the absence of effective peak concentrations could be reasons for the absence of a detectable biomass response.

Elevated O₃ reduced the positive effects of N on greenness of the canopy (NDVI) through accelerated leaf senescence. This effect on leaf life span may be manifested as growth reductions in the longer term as a result of continuous reductions in C assimilation.

Prolonged treatment of this subalpine grassland will be necessary before drawing firm conclusions related to possible interactive effects of increased N input and elevated O₃.

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Supplementary Material

The following supplementary material is available for this article online:

Fig. S1 Means (\pm SE) of total above-ground dry matter (g m^{-2}) and dry matter of the functional groups (grasses, forbs, and sedges) in the years 2005 (a) and 2006 (b). Back-transformed values from ANOVA are shown. Letters indicate significant differences at $P < 0.05$ (Tukey test for multiple comparisons) among the N treatments within each functional group.

Table S1 List of species recorded by point-quadrat method in the *Geo-Montani-Nardetum* monoliths exposed in the fumigation experiment on Alp Flix, 2000 m asl

Table S2 Effects of O₃, N, and year on harvested above-ground biomass of grasses, forbs and sedges in monoliths of a free-air fumigation experiment at Alp Flix in 2004–06

This material is available as part of the online article from: <http://www.blackwell-synergy.com/doi/abs/10.1111/j.1469-8137.2007.02140.x>
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Supplementary Material

Table S1 List of species recorded by point-quadrat method in the *Geo-Montani-Nardetum* monoliths exposed in the fumigation experiment on Alp Flix, 2000 m asl

Species name	
<i>Achillea millefolium</i> L.	<i>Hieracium hoppeanum</i> Schult.
<i>Agrostis capillaris</i> L.	<i>Homogyne alpina</i> (L.) Cass.
<i>Agrostis rupestris</i> All.	<i>Hypochaeris uniflora</i> Vill.
<i>Alchemilla fissa</i> aggr.	<i>Leontodon helveticus</i> Mérat
<i>Alchemilla glabra</i> aggr.	<i>Leontodon hispidus</i> L.
<i>Alchemilla hybrida</i> L.	<i>Leucanthemum vulgare</i> Lam.
<i>Alchemilla xanthochlora</i> aggr.	<i>Ligusticum mutellina</i> (L.) Crantz
<i>Androsace chamaejasme</i> Wulfen	<i>Loiseleuria procumbens</i> (L.) Desv.
<i>Anemone narcissifolia</i> L.	<i>Lotus alpinus</i> (DC.) Rämönd
<i>Antennaria dioica</i> (L.) Gaertn.	<i>Luzula multiflora</i> (Retz.) Lej.
<i>Anthoxanthum alpinum</i> Á. & D. Löve	<i>Myosotis alpestris</i> F. W. Schmidt
<i>Anthyllis vulneraria</i> L.	<i>Nardus stricta</i> L.
<i>Arnica montana</i> L.	<i>Nigritella nigra</i> auct.
<i>Aster bellidiastrum</i> (L.) Scop.	<i>Pedicularis tuberosa</i> L.
<i>Bartsia alpina</i> L.	<i>Phleum rhaeticum</i> (Humphries) Rauschert
<i>Biscutella laevigata</i> L.	<i>Phyteuma orbiculare</i> L.
<i>Briza media</i> L.	<i>Plantago alpina</i> L.
<i>Botrychium lunaria</i> (L.) Sw.	<i>Plantago atrata</i> Hoppe
<i>Campanula barbata</i> L.	<i>Poa alpina</i> L.
<i>Campanula scheuchzeri</i> Vill.	<i>Polygonum viviparum</i> L.
<i>Cerastium fontanum</i> Baumg.	<i>Potentilla aurea</i> L.
<i>Carex sempervirens</i> Vill.	<i>Potentilla erecta</i> (L.) Raeusch.
<i>Carex caryophyllea</i> Latourr.	<i>Primula farinosa</i> L.
<i>Carex ericetorum</i> Pollich	<i>Primula integrifolia</i> L.
<i>Carex montana</i> L.	<i>Pulmonaria australis</i> (Murr) W. Sauer
<i>Carex ornithopoda</i> Willd.	<i>Pulsatilla alpina</i> (L.) Delarbre subsp. <i>apiifolia</i> Nyman
<i>Carlina acaulis</i> L.	<i>Pulsatilla vernalis</i> (L.) Mill.
<i>Centaurea nervosa</i> Willd.	<i>Ranunculus villarsii</i> DC.
<i>Cerastium alpinum</i> L.	<i>Salix</i> spec.
<i>Coeloglossum viride</i> (L.) Hartm.	<i>Salix herbacea</i> L.
<i>Crepis aurea</i> (L.) Cass.	<i>Scabiosa columbaria</i> L.
<i>Crepis pontana</i> (L.) Dalla Torre	<i>Selaginella</i> spec.
<i>Crocus albiflorus</i> Kit.	<i>Silene nutans</i> L.
<i>Daphne striata</i> Tratt.	<i>Soldanella alpina</i> L.
<i>Elyna myosuroides</i> (Vill.) Fritsch	<i>Solidago virgaurea</i> L. subsp. <i>minuta</i> (L.) Arcang.
<i>Euphrasia minima</i> Schleich.	<i>Thesium alpinum</i> L.
<i>Lloydia serotina</i> (L.) Rchb.	<i>Thymus serpyllum</i> aggr.
<i>Festuca violacea</i> Gaudin	<i>Tofieldia</i> spec.
<i>Galium anisophyllum</i> Vill.	<i>Trifolium alpinum</i> L.
<i>Gentiana acaulis</i> L.	<i>Trifolium pratense</i> L. subsp. <i>nivale</i> (W. D. J. Koch) Arcang.
<i>Gentiana campestris</i> L.	<i>Trollius europaeus</i> L.
<i>Gentiana verna</i> L.	<i>Vaccinium myrtillus</i> L.
<i>Geum montanum</i> L.	<i>Vaccinium uliginosum</i> L.
<i>Gymnadenia conopsea</i> (L.) R. Br.	<i>Vaccinium vitis-idaea</i> L.
<i>Helictotrichon versicolor</i> (Vill.) Pilg.	<i>Viola calcarata</i> L.
<i>Helianthemum nummularium</i> (L.) Mill.	

Table S2 Effects of O₃, N, and year on harvested aboveground biomass of grasses, forbs, and sedges in monoliths of a free-air fumigation experiment at Alp Flix in 2004–06

	Grasses dry matter (g m ⁻²)			Forbs dry matter (g m ⁻²)			Sedges dry matte (g m ⁻²) r		
	N df	D df	<i>F</i>	N df	D df	<i>F</i>	N df	D df	<i>F</i>
Block	2	4	2.58	2	4	6.63	2	4	1.91
O ₃	2	4	0.78	2	4	0.89	2	4	1.18
N	1	167	2.83	1	169	5.77*	1	169	39.9***
N × N	1	167	1.30	1	169	2.58	1	169	0.02
N × O ₃	2	167	0.85	-	-	-	-	-	-
Year	2	344	112.28***	2	348	51.38***	2	352	101.04***
Year × Block	4	344	3.39**	4	348	9.32***	4	352	3.66**
Year × O ₃	4	344	1.37	4	348	3.98**	-	-	-
Year × N	2	344	0.83	2	348	3.22*	2	352	40.46***
Year × N × O ₃	4	344	2.35	-	-	-	-	-	-

Numerator (N df) and denominator degrees of freedom (D df) and *F*-values are displayed.

Terms with ‘-’ were not fitted in the particular model. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Fig. S1. Means (\pm SE) of total aboveground dry matter [g m^{-2}] and dry matter of the functional groups (grasses, forbs, and sedges) in the years 2005 (a) and 2006 (b). Back transformed values from ANOVA are shown. Letters indicate significant differences at $P < 0.05$ (Tukey test for multiple comparisons) among the N treatments within each functional group.

