Anthropogenic ozone and nitrogen deposition:

Effects on flower production and flowering phenology of 12 alpine species

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Abstract

In the next decades, natural ecosystems will face increasing immissions of air pollutants, such as ozone and nitrogen. Both substances are expected to have major impacts on plants, as ozone is known to be phytotoxic and nitrogen, an important and often limiting element of plant nutrition, may stimulate growth. Little is known about the response of alpine grassland communities to elevated ozone concentrations and even less is known about a potential interaction with increased nitrogen deposition. Therefore, a long-term experiment was initiated in 2004 in the Swiss Alps at 2000 m a.s.l., using monoliths of an alpine grassland. The monoliths were exposed to combined elevated nitrogen (ambient, +5, +10, +25, +50 kg N ha^{-1} yr^{-1}) and ozone concentrations (ambient (~ 40 ppb), 1.5 x ambient, 2 x ambient concentration) using a free-air fumigation system with nine fumigation rings. Nitrogen was applied in biweekly intervals by irrigation with ammonium nitrate. The current study was carried out in the third experimental year and focused on the effects of ozone and nitrogen on flower production and flowering phenology. Sexual reproduction enables plants to colonize new habitats and adapt to new environmental stresses – two important features in the light of expected climate change. Species abundance, flower production, and flowering phenology of 12 selected key species were recorded. Microclimatic parameters (temperature and evaporation) and soil water content were surveyed to separate the treatment impact from possible microclimatic site effects. Ozone and nitrogen effects and its interactions were analysed using a split-plot ANOVA.

Of the 12 species under investigation only two, namely *Carex sempervirens* (sedge) and *Helictotrichon versicolor* (grass), were negatively affected by ozone, the latter only marginally significant. Flower production of *C. sempervirens* was reduced by 50% in the highest ozone treatment. Nitrogen did not significantly stimulate any species' flower production or flowering phenology, but *Festuca violacea* (grass) tended to produce more inflorescences in the intermediately fertilized plots. Flower development of *Potentilla aurea* (forb) was significantly retarded for a few days in the high nitrogen treatment. In terms of species abundance, *C. sempervirens* was the only species showing a strong positive reaction to nitrogen application. Two species showed significant ozone x nitrogen interactions, *Ranunculus villarsii* (forb) in terms of flower production and flowering phenology and *Nardus stricta* (grass) in terms of species abundance. The direction of the interaction could not be determined, as effects in the 1.5 x ambient and 2 x ambient ozone treatments were opposing.

Microclimate varied between the nine fumigation rings with temperature differences of 1°C and approximately 10% variability in evaporation and soil water content. Four fumigation rings were warmer, drier, and showed higher evaporation than the average of all rings, and three rings were cooler, wetter, and showed less evaporation. However, the recorded temperature differences had no effect on flower production and development, and temperature variability did not superpose a possible ozone effect.

The observed effects of ozone and nitrogen on flower production and phenology were smaller than expected. Three years of simulated environmental changes might have been be too short for detecting strong effects on species of a stress-tolerant alpine plant community. To understand the complex interactions of air pollutants on alpine grassland, further research is needed.

Abbreviations

AA	ambient air
ANOVA	analysis of variance
ART	Agroscope Reckenholz-Tänikon Research Station ART
CO	carbon monoxide
D	day
ETH	Swiss Federal Institute of Technology
FFGS	fraction of flowering generative shoots
FGS	fraction of generative shoots
GR	Graubünden, Grison
N	nitrogen
NAS	number of aboveground shoots
NDVI	normalized difference vegetation index
NGS	number of generative shoots
NH_x	reduced nitrogen
NI/PQH	number of inflorescences per point-quadrat hits
NMVOCs	non-methane volatile organic compounds
NO_x	nitrogen oxides
O_3	ozone
PQH	point-quadrat hits
RS	reproductive structures
SWC	soil water content

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1 Introduction

Biodiversity of alpine ecosystems is particularly endangered by interactions of climate change, nitrogen deposition, and land-use changes (Sala et al., 2000). Tropospheric ozone, a phytotoxic substance, might additionally harm this ecosystem. Due to long-range transport and delayed decomposition, ozone concentrations are often even higher in rural than in urban areas. Ozone is produced in the troposphere in a complex chain of reactions that involve the ozone precursors nitrogen oxides (NO_x), non-methane volatile organic compounds (NMVOCs), and carbon monoxide (CO). The main sources of those precursor substances are fossil fuel combustion, road transport, natural and anthropogenic soil release (NO_x), biomass burning, and use of solvents (NMVOCs) (IPCC, 2000). NO_x do not only act as ozone precursor substances but are also deposited, together with NH_x (mainly emitted by intensive agriculture), in large amounts into ecosystems. Efforts have been made to reduce air pollutants, but scenarios of future global emissions predict a further increase of NO_x until 2020 and an increasing emission of NMVOC until 2050 (IPCC, 2000).

Elevated ozone concentrations have been shown to affect various parts and processes of plants growing in (semi-)natural vegetation, including leaf injury, reduction in vegetative and reproductive growth, and altered resource allocation to roots, aboveground shoots, and reproductive organs (Davison & Barnes, 1998). The underlying physiological mechanisms are quite complex. Briefly, ozone enters the plant via stomata and reacts as a potent oxidant with cellular components, eventually leading to damaged membranes and/or cell death. Carbon assimilation may decrease as the activity of the enzyme Rubisco (catalysing carbon fixation inside chloroplasts) is reduced and its concentration lowered (due to damaged chloroplast or photosynthetic cells). Repair of the damaged membranes may consume additional carbon resources. Ozone additionally impairs phloem loading, resulting in increased concentrations of soluble and insoluble carbohydrates in leaves, and decreased carbon allocation to roots (for further details see Fuhrer & Booker, 2003 and Andersen, 2003).

Like ozone, gaseous nitrogen pollutants can act as phytotoxic substances, but concentrations in rural areas are relatively low and therefore pose no major threat to plants of (semi-)natural vegetation (Bobbink, Hornung & Roelofs, 1998). More important is the nitrogen deposition onto soils, influencing plants indirectly by changing soil characteristics. Depending on the nutrient status and buffering capacity of the soil, elevated nitrogen deposition may enhance nutrient availability, change nitrogen and carbon cycling in soils, and lead to acidification, resulting in leaching of essential cations and higher concentrations of toxic metals. Plants may react in different ways to altered soil conditions. Nitrogen, contained in amino acids, proteins, and DNA, is an essential element of plant nutrition. Thus, enhanced nitrogen availability commonly leads to increased growth, whereas acidification can have the opposite effect (Throop & Lerdau, 2004). Plants may react to enhanced nitrogen availability by increasing the nitrogen content of plant tissue (thus lowering the C:N ratio, as reported by Pitcairn, Fowler & Grace, 1995). A lower C:N ratio is known to increase plants susceptibility to stress, like herbivory, frost, and drought (Throop & Lerdau, 2004; Sitte et al., 2002). Finally, the sum of these effects may alter plant competition and lead to changes in species composition. Natural ecosystems are particularly affected, since they are mostly nitrogen limited and its species, adapted to nutrient-poor conditions, might not compete successfully on nitrogen richer soils, resulting in crowding out of those species (Bobbink, Hornung & Roelofs, 1998).

Few experiments have investigated the interacting effects of ozone and nitrogen on plants and

most studies dealt with cultivated plants or trees (e.g. Adaros, Weigel & Jager, 1991; Pell et al., 1990; Calatayud, Pomares & Barreno, 2006; Utrianien & Holopainen, 2001). The few experiments using grassland species revealed contrasting results. Whitfield, Davison & Ashenden (1998) claimed that high nutrient supply protected *Plantago major* plants against ozone damage, whereas most authors did not find an ozone x nitrogen interaction (Thwaites, 1997; Bass et al., 2006; Samuelsson, Peacock & Barnes, 2006). The effects of co-occurring ozone and nitrogen deposition on alpine grassland species have not been investigated thus far.

The current study focused on the effects of ozone and nitrogen on flower production and flowering phenology of alpine plants. Twelve selected key species of a *Hypochoero-Nardetum* grassland were investigated. The *Hypochoero-Nardetum* grassland is a typical plant community on acidic soils of extensively used meadows at high elevation (Dietl, 1995; 1997). It is mainly dominated by *Nardus stricta*, *Festuca violacea*, and *Carex sempervirens*, but contains as much as 90 different vascular plant species. Alpine plants show variable phenotypic responses to environmental stresses. Most species show a stronger plasticity in terms of sexual rather than vegetative reproduction (Kay, 1987). For the survival of alpine grassland species, reproductive success is crucial. Although up to 90% of alpine plants are able to reproduce clonally (Klimes et al., 1997), sexual reproduction plays an important role in colonizing new sites and maintaining genotypic variability, a prerequisite for adaptation to new selective pressures (e.g. climate change). In harsh alpine environments with short growing seasons, alternation of flowering phenology might impair pollination and seed maturation, leading to reduced success of sexual reproduction.

The effects of ozone on reproductive development of cultivated crops or ornamental plants have been relatively well examined (see Black et al., 2000 and literature cited therein), but less research has been conducted on the reaction of species growing in (semi)-natural vegetation. Exceptions are studies on *Plantago major* (Lyons & Barnes, 1998), *Apocynum androsaemifolium*, (Bergweiler & Manning, 1999), *Trifolium* spp. (Gimeno et al., 2004), *Spartina alterniflora* (Taylor et al., 2002), 17 native herbaceous species (Bergmann, Bender & Weigel, 1996), *Phleum* spp. (Danielsson, Gelang & Pleijel, 1999), wetland plants (Franzaring et al., 2000), and meadow species (Rämö et al., 2006). Reactions to ozone range from decreased, to not affected, to increased flower production. The experiments on timing of flowering also revealed contrasting results. Currently, two hypotheses are proposed to explain ozone effects on flowering and seed production: First, ozone directly affects the generative tissue resulting in reduced growth of reproductive structures (Bosac et al., 1994). Second, ozone decreases the plants carbon resources and changes assimilate transport, leading to reduced allocation to reproductive structures (Black et al., 2000).

In contrast, nitrogen generally had a stimulating effect on flower production of high elevation plants (Muñoz et al., 2005; Soudzilovskaia & Onipchenko, 2005; Leith et al., 1999; Kellner, 1993; Power et al., 1995). However, some authors reported no reaction to elevated nitrogen (Leith et al., 1999; Calvo et al., 2005) and others observed negative effects on flowering (Soudzilovskaia & Onipchenko, 2005). Increased nitrogen concentrations in soils may result in better availability and enhanced root-uptake of nitrogen, allowing the plant to invest additional nitrogen in growth of new structures, including reproductive organs.

The effects of co-occurring ozone and nitrogen on flower production and flowering phenology have not yet been investigated. However, high-nutrient growing conditions seemed to prevent negative effects of ozone on seed production of an ozone-sensitive line of *Plantago major* (Whitfield, Davison & Ashenden, 1998).

In summary, there is a general lack of knowledge concerning the interactive effects of ozone and nitrogen deposition, especially on sexual reproduction of plants growing in (semi-)natural vegetation such as alpine pastures. To address this gap of knowledge, a survey was carried out in the long-term free-air ozone fumigation experiment maintained by Agroscope Reckenholz-Tänikon Research Station (ART) at Alp Flix GR, Switzerland (2000 m a.s.l.), where a species-rich alpine pasture was exposed to simulated future concentrations of ozone and nitrogen.

The aims of this study were to investigate the effects of elevated ozone and nitrogen deposition on (a) flower production, as an indicator for plants investments in sexual reproduction and (b) flowering phenology of 12 selected key species growing in an alpine pasture. An additional aim was to (c) analyse possible unintended microclimatic impacts resulting from the heterogeneous environment of the experimental site.

Ozone was expected to decrease flower production and delay flowering, whereas nitrogen was expected to increase flower production and accelerate phenological development of the selected key species. Furthermore, ozone was expected to diminish the stimulating effect of nitrogen on flowering.

2 Methods

2.1 Experimental setup

The study was carried out at Alp Flix, Sur (GR), Switzerland (2000 m a.s.l.), where a free-air fumigation system had been installed in 2004. Alp Flix is a high altitude plain covered by extensively managed meadows and pastures at 100 m below the climatic tree line with mean annual temperature of approximately 2.2°C. Precipitation peaks in summer with an annual sum of approximately 1050 mm. The length of the growing season varies between 20 and 24 weeks. The fumigation study was designed as a multi-year experiment providing realistic conditions. A total of 180 monoliths (30 x 40 x 20 cm) were excavated from a more than 60 year old *Hypochoero-Nardetum* pasture and placed in plastic boxes. Approximately 90 vascular plant species were observed on the excavated turfs, with three dominant species: *Nardus stricta*, *Festuca violacea* (grasses), and *Carex sempervirens* (sedge). Forbs accounted for about three-quarters of the species diversity, whereas one ninth were grasses, and only a few sedge species were found. The investigated *Hypochoero-Nardetum* grassland is a typical plant community of extensively used meadows at high elevations (Dietl, 1995, 1997). Like in most alpine ecosystems, soil characteristics are very heterogeneous and species show a patchy distribution. The soil is a slightly acidic (pH 4.8 - 6) cambisol developed on serpentinite bedrock. Soil depth varies between 20 cm and 40 cm, but rooting depth is limited to the upper 15 - 20 cm.

The 180 boxes were placed in groups of 20 in shallow pits flush with the surrounding grassland inside nine circular fumigation rings and exposed to different concentrations and combinations of nitrogen and ozone, using a split-plot experimental design. Three ozone treatments replicated three times were applied: ambient air (AA; ~ 40 ppb), 1.5 x ambient (1.5 x AA; ~ 60 ppb), and 2 x ambient (2 x AA; ~ 80 ppb). The elevated ozone concentrations correspond to projected future 1h mean ozone values for the year 2050 and 2100, respectively, obtained from interpolation of long-term records of 1h mean ozone values from Davos (GR), a similarly situated air quality monitoring station. The nine fumigation rings were installed along a small ridge and were grouped into three blocks. Each block contained one of the three ozone treatments. With ozone

concentrations at these altitudes being mainly controlled by the global background concentration, the ozone concentrations were increased during the day as well as the night. For details concerning the free-air fumigation system see Volk et al. *(*2003).

During the growing season, atmospheric nitrogen deposition was simulated by applying a bi-weekly irrigation with a solution of ammonium nitrate in 200 ml of well water. The additional input amounts to +5, +10, +25, and +50 kg N ha^{-1} yr^{-1}, (subsequently referred to as N0 (= control treatment with pure well water), N5, N10, N25, and N50). The highest nitrogen treatment applied corresponds to the peak deposition measured on the Swiss Central Plateau (Rihm & Kurz 2001). The additional water supply is equivalent to only about 2% of the annual rainfall and the nitrogen contained in the well water amounts to less than 0.05 kg N ha^{-1} yr^{-1} applied to the plots. Wet and dry deposition of NH_3 and NO_2 were monitored at the site. Based on preliminary estimates, the background nitrogen load is 6 - 8 kg N ha^{-1} yr^{-1}. To minimize microclimatic site effects, the monoliths were rearranged each year, receiving the same ozone and nitrogen treatment within a different fumigation ring.

2.1.1 Microclimatic variability between fumigation rings

In order to separate the treatment impact from microclimatic influences, soil temperature, evaporation, and soil water content were recorded. Date of snow melt, one of the most important factors for phenological development (Totland & Alatalo, 2002), was standardized by removing snow from all plots on April 20, 2006, when the first snow-free patches were observed on the experimental site. Snow that fell after April 20 melted within short time. Snow-cover was therefore not considered a microclimatic factor varying between fumigation rings.

On April 25, 2006, temperature loggers (HOBO Pendant, Onset, USA), recording soil temperature every 60 minutes, were buried 5 cm under ground, two in each fumigation ring, 50 cm south- and northwards of the monolith-boxes. Data was read out from all loggers on August 21, 2006. One logger (buried in ring 6) did not work properly, so there data was obtained from one single logger.

Evaporation was measured on July 5, 2006, a sunny and windy day. The aim was to obtain relative evaporation differences between the fumigation rings, resulting from different exposition to wind and solar radiation, assuming that the relative differences do not change in the course of the growing season. In every ring, five Petri dishes filled with four wet filtering papers (12.5 cm diameter) were exposed to direct sun for approximately one hour. The Petri dishes were weighed before and after exposure and the exact time of exposure was recorded. The procedure was repeated every second hour, totally 5 times. To assure an average inclination for each fumigation ring and to provide a similar substrate, a wooden board (2 x 0.5 m) was put inside every ring where the Petri dishes were put on. The average amount of evaporated water per dish and ring was calculated for all five measuring periods. In the intervals between two measurements, evaporation was interpolated. Finally, a daily sum of evaporating water was calculated. Unfortunately, the board in ring 8 was slightly darker than the other ones. Therefore it absorbed more radiation and was warmer, especially in the evening, resulting in a comparatively high evaporation.

Soil water content was measured with Time Domain Reflectometers (TDR) in regular intervals of 5 - 10 days in all the monoliths of the nitrogen +10, +50 kg N ha^{-1} yr^{-1}, and the control treatment (Volk, unpublished data). Inclination and exposition of each fumigation ring were measured by inclinometer and compass, respectively. Temperature, being highly correlated with evaporation and soil water content and directly influencing both parameters, was used as an

indicator of microclimate for subsequent analysis.

2.2 Analyses

Flower production and species abundance

Twelve commonly present species were included in the analysis (Table 1): four grass species (*Briza media, Festuca violacea, Helictotrichon versicolor, Nardus stricta*), one sedge (*Carex sempervirens*), and seven herbaceous species (*Arnica montana, Gentiana acaulis, Leontodon helveticus, Ligusticum mutellina, Potentilla aurea, Ranunculus villarsii, Trifolium alpinum*). The selected species belong to the 15 most abundant species found in the investigated pasture, accounting for over 50% of the vegetation cover.

Data was collected during the 2006 growing season, in the third year of the experiment. In order to obtain the fraction of shoots producing flowers, the total number of aboveground shoots (NAS) of all herbaceous species was counted in each monolith. The number of generative shoots (NGS) with their phenological phase (unopened buds, open flowers, and withered flowers) was recorded in weekly intervals, starting when the first generative shoots appeared. The fraction of generative shoots (FGS) was calculated by dividing NGS by NAS. *Potentilla aurea* is the only analysed herbaceous species producing several flowers per generative shoot. All generative shoots of *P. aurea* were marked and the number and phenological phase (unopened buds, open flowers, and withered flowers) of all reproductive structures (RS) present on the shoot were recorded in weekly intervals.

Distinguishing genetic individuals from aboveground observations is difficult for alpine plant species, since most species show some kind of clonal propagation and form more than one shoot per individual. Therefore, aboveground shoots were used as observation unit for all herbaceous species, including sexually immature shoots, since no visual distinction between mature and immature shoots could be made.

It was not possible to count single shoots of grasses and sedges, as the species could not be identified for every single shoots. As a measure of abundance, data from point-quadrat sampling were used (Bassin, unpublished data). Thereby, a customized point-quadrat frame of 18 intersecting points was used (Stampfli, 1991). Thus, for every species the maximum number of point-quadrat hits (PQH) per monolith was 18. The number of inflorescences (NI) of the sedge and grass species was counted at time of maximum flowering. For the analysis, a ratio between NI and PQH was calculated for each species (NI/PQH).

Flowering phenology

Phenological development was only analysed for herbaceous species, as visual distinction of reproductive development in graminoids is difficult. As an indicator for flowering phenology, repeated measurements of the fraction of flowering generative shoots (FFGS) were used. The FFGS was obtained by dividing the number of shoots carrying open or withered flowers by the total number of generative shoots. Additionally, the fraction of flowering reproductive structures was calculated for *P. aurea* by dividing the number of open or withered flowers by the maximal number of reproductive structures ever present on a shoot during the measuring period.

As an additional measure of microclimatic influences on phenological development of plants,

canopy development was surveyed in every monolith by normalized difference vegetation index (NDVI) measurements in 1 - 3 weeks intervals. NDVI is an integrated measure of leaf area index (LAI) and chlorophyll content of the vegetation, in other words the greenness of the canopy (Tucker, 1979). A multispectral radiometer (MSRSYS16R System, Cropsan Inc., Rochester, USA), measuring the vegetations absorbance of radiation at wavelengths 810 and 680 nm, was placed 90 cm above the monoliths. Values for single monoliths were obtained by covering the surrounding vegetation with black cloth.

The NDVI was calculated as follows: NDVI = $\frac{(\rho 810 - \rho 680)}{(\rho 810 + \rho 680)}$

2.3 Statistics

The results of the four pseudo-replicated plots within each of the fumigation rings were pooled to an average, which was used for all subsequent analyses (thus, n = 45). To meet the assumptions of an ANOVA (normal distribution and homogeneity of variance), the FGS-data was logit-transformed. If data contained zero-values (data of *P. aurea* and *R. villarsii*) a constant (k = 0.5) was added to all values before transformation. The NI/PQH-data were Box-Cox-transformed (with lambda = 0.5 for *B. media*, *F. violacea*, and *N. stricta* and lambda = 0 for *C. sempervirens* and *H. versicolor*). If zero-values appeared in the PQH-data, they were replaced by a minimal value of one hit per four pooled pseudo-replicated plots, resulting in an averaged value of 0.25 (two values of *B. media* and 4 values of *H. versicolor*). Also zero-values within the NI-data were replaced by a value of 0.25 (eight values of *C. sempervirens* and three values of *H. versicolor*). For the FFGS-data, no transformation was needed.

The treatment effects were tested by means of a split-plot analysis of variance (ANOVA) with ozone on the main plot level and nitrogen on the sub-plot level. Statistical analysis was carried out in SAS and R statistical package. A linear mixed model design was used for the ANOVA and analysed in the SAS procedure Proc Mixed (see Littell, Pendergast & Natarajan, 2000 and Littell et al., 1996). Intercept was treated as random effect, block, ozone, and fumigation ring were treated as class variables. For analysis of species abundance (NAS and PQH) and flower production (FGS and NI/PQH) following factors were tested (with O_3 = ozone and N = nitrogen): Block, O_3, N, {N x N}, {O_3 x N}, {O_3 x N x N}. A model of the original data, consisting of the best fitting factor combination, was obtained by means of model reduction (using likelihood ratio tests), and was plotted thereafter.

The phenological development (FFGS) was analysed by repeated measures ANOVA assuming a compound symmetric covariance structure, (which specifies that repeated observations on the same subject have homogenous covariance and variance). Block, ozone, and fumigation ring were treated as class variable. Model reduction was carried out using following effects for the full model (with O_3 = ozone, N = nitrogen, and D = Day):
Block, O_3, N, {N x N}, D, {D x D}, {O_3 x N}, {O_3 x N x N}, {O_3 x D}, {O_3 x D x D}, {N x D}, {N x D x D}, and {O_3 x N x D}. Modelled data was plotted.

Microclimatic influences were tested by correlating the average temperature of every fumigation ring with the averaged measure for flower production and flowering phenology, respectively.

Table 1. Flowering and growth characteristics of the 12 species analysed and the parameters measured.

	Functional group	Growth form[a]	Flowering type[b]	Time of full flowering[c]	Flowering intensity	Parameters measured[d]					
						NAS	PQH	NI	NGS	FFGS	RS
Arnica montana	Forb	R	SF	late	very low	X			na	na	
Briza media	Grass	MS	SP	late	high		X	X			
Carex sempervirens	Sedge	T	MT	mid season	low		X	X			
Festuca violacea	Grass	T	MT	late	inter-mediate		X	X			
Gentiana acaulis	Forb	R	SF	early	very low	X			na	na	
Helictotrichon versicolor	Grass	MS	SP	late	very high		X	X			
Leontodon helveticus	Forb	R	SF	late	inter-mediate	X			X	X	
Ligusticum mutellina	Forb	SL	U	late	very low	X			na	na	
Nardus stricta	Grass	T	MT	late	high		X	X			
Potentilla aurea	Forb	MS	MF	mid season	low	X			X	X	X
Ranunculus villarsii	Forb	SS	SF	mid season	inter-mediate	X			X	X	
Trifolium alpinum	Legume	SS	MF	late	very low	X			na	na	

[a] R = rosette, MS = multiple shoots emerging closely together from below- or aboveground stem or root, T = tussock, SL = single leaves emerging from belowground root, SS = separated single shoots emerging from belowground root.

[b] SF = single flower or flower head per generative shoot, SP = single spike per shoot, MT = multiple inflorescences per tussock, U = umbel, MF = multiple flowers per generative shoot.

[c] Date of full flowering: early = week 21 - 22, (22.05 - 04.06.06); mid season = week 23 - 24, (05.06. - 18.06.06); late = week 25 - 27, (19.06. - 09.07.06).

[d] NAS = number of aboveground shoots, PQH = point-quadrat hits, NI = number of inflorescences, NGS = number of generative shoots, FFGS = fraction of flowering generative shoots, RS = number of reproductive structures, X = parameter measured and analysed, na = parameter measured but not included in analysis because of very low flowering intensity.

3 Results

3.1 Microclimatic variability between fumigation rings

Within the measuring period (28.4.2006 – 20.8.2006), soil temperature at –5 cm varied from 12.1°C during day (8 a.m. – 8 p.m.) to 10.3°C during night (8 p.m. – 8 a.m.). Temperatures below 0°C were not observed during this period. The overall average soil temperature was 11.2°C, the average soil water content (SWC) was 23.9%, and the average amount of water evaporated on July 5, 2006 was 42.9 g d^{-1}. Microclimate varied between the nine fumigation rings, with temperature differences of more than 1°C and approximately 10% difference in evaporation (Table 2). Soil water content also varied in a range of about 10%. These differences are likely due to inhomogeneous inclination and orientation of the plots. Temperature was highly correlated with SWC and evaporation (Fig. 1: A and B), but no significant correlation was found between evaporation and SWC (Fig. 1: C). Rings 3, 6, 8, and 9 were warmer than the average of all rings, had a higher evaporation, and a drier soil. Rings 1, 2, and 4 were cooler, had a lower evaporation, and a wetter soil. Rings 5 and 7 did not show a consistent microclimatic picture. Although ozone treatments were randomly assigned to fumigation rings, there was strong variation in average temperature, evaporation, and SWC among ozone treatments (Fig. 1: D).

Table 2. Microclimatic parameters (average temperature and evaporation), average soil water content (SWC), and exposition (inclination and slope orientation) of the nine fumigation rings.

	Ozone treatment	**Temperature** °C	**Evaporation** g d^{-1}	**SWC** %	**Inclination** %	**Slope orientation** ° clockwise deviation from North
mean	**-**	**11.2**	**42.9**	**23.9**	**-**	**-**
Ring 1	1.5 x AA	10.7	42.4	24.5	5	118
Ring 2	2 x AA	10.6	40.8	24.9	10	174
Ring 3	AA	11.5	42.6	22.3	9	51
Ring 4	1.5 x AA	10.8	42.1	26.0	10	124
Ring 5	2 x AA	11.4	42.0	24.7	6	124
Ring 6	AA	11.8	44.0	22.7	-3	17
Ring 7	AA	11.1	42.2	23.1	8	124
Ring 8	1.5 x AA	11.4	45.3*	23.0	10	107
Ring 9	2 x AA	11.6	44.8	23.5	-1	79

* The recorded evaporation of ring 8 might be too high (see section 2.1.1.).

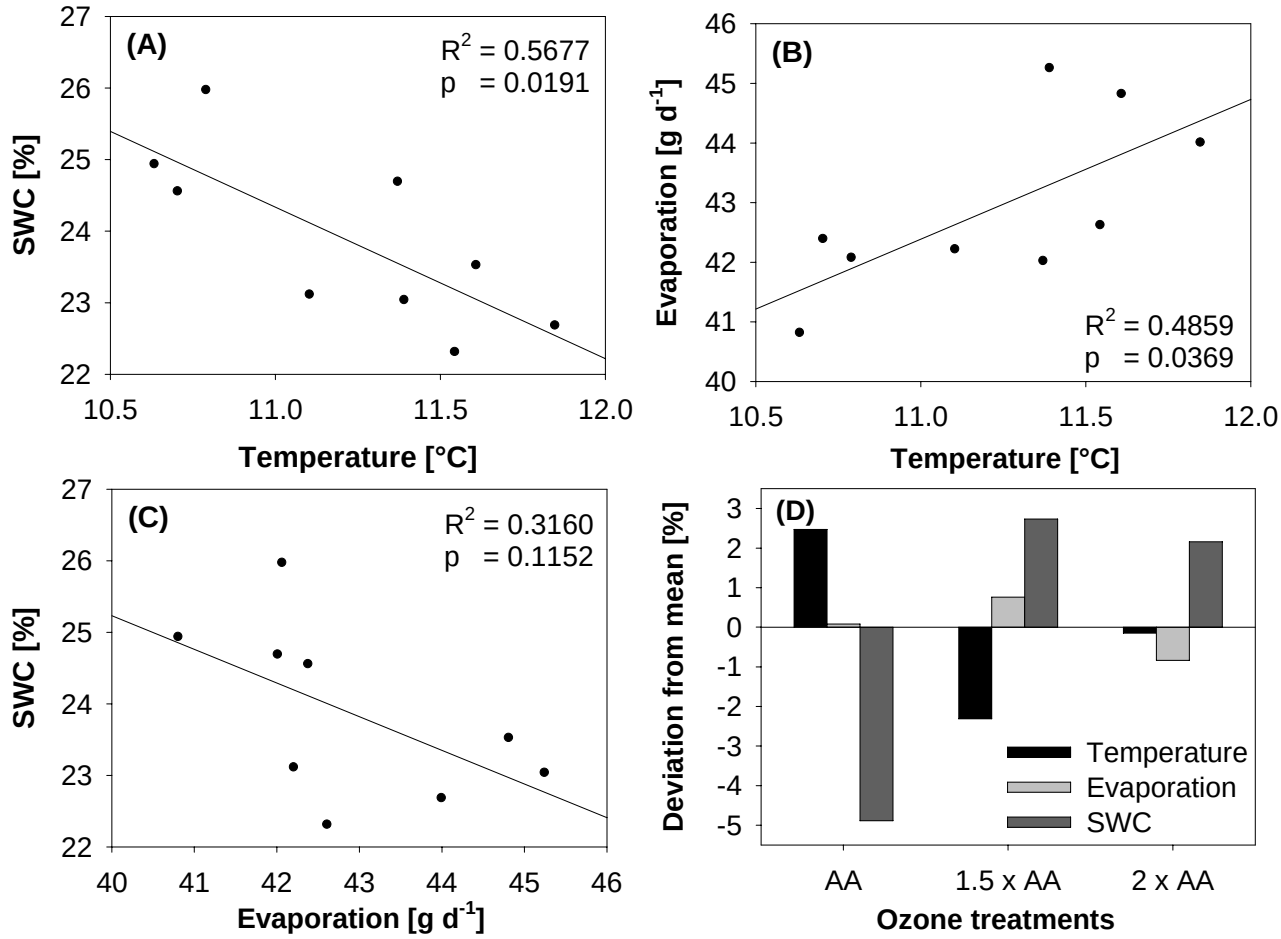

Fig. 1. Correlation of **(A)** mean temperature and mean soil water content (SWC), **(B)** mean temperature and evaporation on July, 5 and, **(C)** mean SWC and evaporation on July, 5. Every data point corresponds to one fumigation ring. **(D)** Deviation of the averaged temperature, evaporation, and SWC of each ozone treatment form the mean values of all nine rings, (in percent of the mean values).

3.2 Effects on species abundance

No ozone effect on species abundance was observed and only *C. sempervirens* reacted strongly to nitrogen deposition (Table 3). Nearly twice as many point-quadrat hits (PQH) were recorded for *C. sempervirens* in the highest nitrogen treatment compared to the control treatment, whereas *T. alpinum* and *A. montana* both showed fewer number of aboveground shoots (NAS) in high nitrogen treatments (not significant). The NAS of *G. acaulis* showed a significant non-linear reaction to nitrogen. No ozone effect was observed on any species' NAS or number of PQH, respectively, but three species (*L. mutellina*, *P. aurea*, and *N. stricta)* showed a significant ozone x nitrogen interaction.

Table 3. Results of split-plot ANOVA of species abundance; NAS = number of aboveground shoots, PQH = point-quadrat hits; (*) p<0.1, * p<0.05, **p<0.01, *** p<0.001, ns = not significant (factor excluded during model reduction)

	Block	O_3	N	N x N	O_3 x N	O_3 x N x N
NAS						
A. montana	0.4699	0.6034	0.0739(*)	ns	ns	ns
G. acaulis	0.3811	0.7434	ns	0.0343*	ns	0.0903(*)
L. mutellina	0.6041	0.5811	0.3752	ns	0.0259*	ns
L. helveticus	0.1416	0.6050	0.6141	ns	ns	ns
P. aurea	0.4682	0.6436	ns	0.3770	ns	0.0128*
R. villarsii	0.4898	0.4525	0.3444	ns	ns	ns
T. alpinum	0.8475	0.6909	0.0697(*)	ns	ns	ns
PQH						
B. media	0.6536	0.7085	0.1265	ns	ns	ns
C. sempervirens	0.3052	0.4411	0.0003***	ns	ns	ns
F. violacea	0.6032	0.6313	0.7978	ns	ns	ns
H. versicolor	0.3894	0.1758	0.2111	ns	ns	ns
N. stricta	0.2389	0.4334	0.6114	0.9071	0.0192*	0.0601(*)

3.3 Effects on flower production

None of the species showed significantly stimulated flower production with nitrogen deposition or higher soil temperatures (Table 5), but flower production of *C. sempervirens* was significantly reduced with elevated ozone (Table 4). This species showed only half the number of inflorescences per point-quadrat hits (NI/PQH) in the elevated ozone treatments compared to the AA treatment (Fig. 2). The NI/PQH of *H. versicolor* also showed a negative, but non-significant reaction to elevated ozone concentrations. A marginally significant non-linear reaction on elevated nitrogen input was recorded for the NI/PQH of *F. violacea*, with a maximal NI/PQH at N25. The fraction of generative shoots (FGS) of *R. villarsii* showed a significant ozone x nitrogen interaction (Fig. 2). Surprisingly, in the AA treatment the FGS of *R. villarsii* did not react on elevated nitrogen deposition, whereas in the 1.5 x AA treatment the FGS showed a negative, in the 2 x AA a positive reaction. The FGS of *L. helveticus* and *P. aurea* and the NI/PQH of *B. media* and *N. stricta* showed no reaction to the ozone nor nitrogen treatment. The number of reproductive structures per generative shoot (RS/GS) of *P. aurea* was not affected by ozone and nitrogen either. Four herbaceous species (*A. montana, G. acaulis, L. mutellina*, and *T. alpinum*) showed a very low flowering intensity and could therefore not be analysed.

Table 4. Results of split-plot ANOVA of flower production. FGS = fraction of generative shoots, NI/PQH = number of inflorescences per point-quadrat hits, RS/GS = reproductive structures per generative shoot; (*) p<0.1, *p<0.05, **p<0.01, *** p<0.001, ns = not significant (factor excluded during model reduction).

	Block	O_3	N	N x N	O_3 x N	O_3 x N x N
FGS						
L. helveticus	0.5849	0.5594	0.8775	ns	ns	ns
P. aurea	0.2180	0.2374	0.1819	ns	ns	ns
R. villarsii	0.4466	0.8200	0.7211	ns	0.0084**	ns
NI/PQH						
B. media	0.8740	0.7742	0.8892	ns	ns	ns
C. sempervirens	0.6712	0.0311*	0.4267	ns	ns	ns
F. violacea	0.4642	0.1457	0.7540	0.0564(*)	ns	ns
H. versicolor	0.9261	0.0539(*)	0.2677	ns	ns	ns
N. stricta	0.2598	0.4311	ns	0.5604	ns	0.1184
RS/GS						
P. aurea	0.8580	0.3593	0.3330	ns	ns	ns

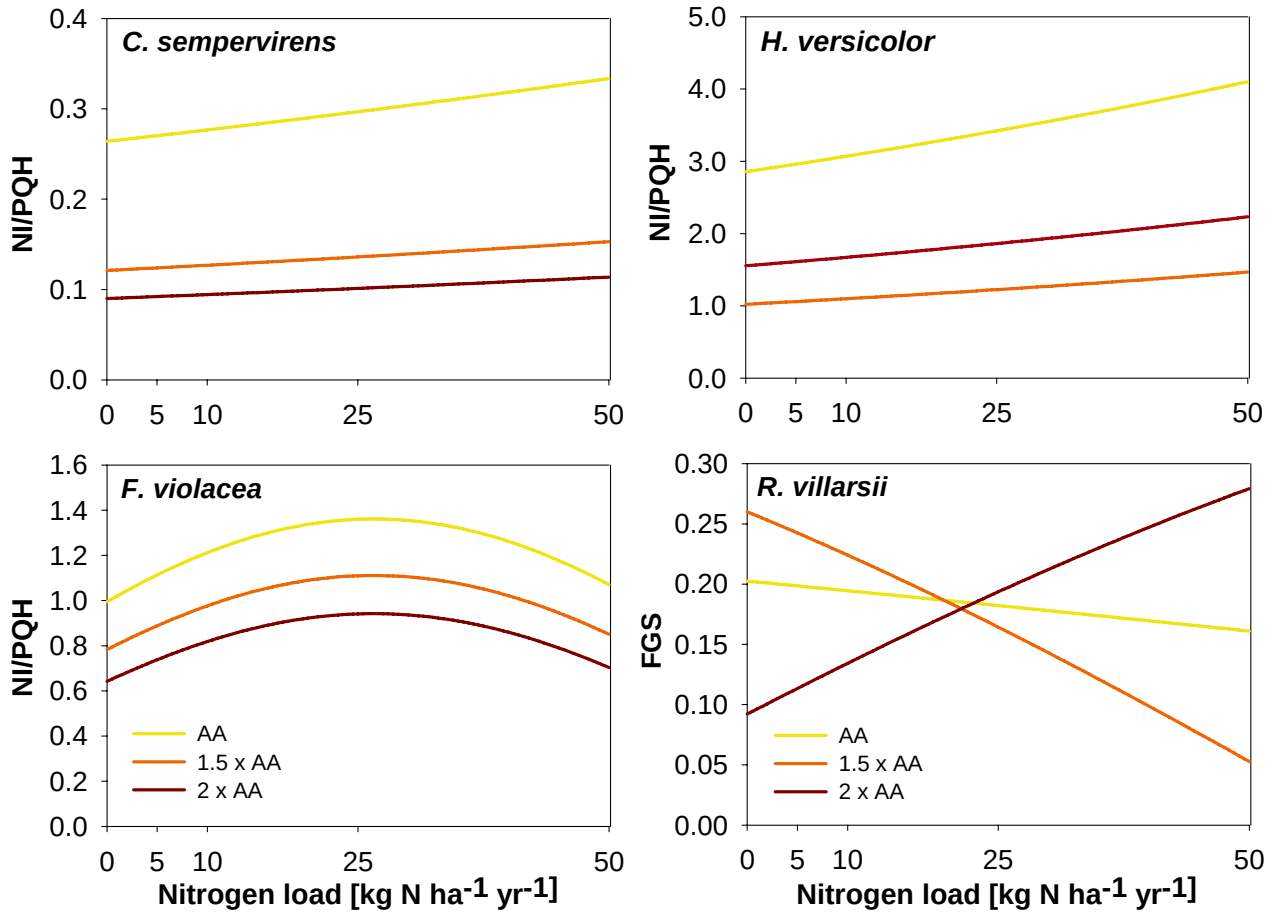

Fig. 2. Effects of ozone and nitrogen on flower production of *C. sempervirens*, *H. versicolor, F. violacea*, and *R. villarsii*, modelled data. NI/PQH = number of inflorescences per point-quadrat hits, FGS = fraction of generative shoots, AA = ambient air ozone concentration.

Table 5. Correlation of temperature and flower production. FGS = fraction of generative shoots, NI/PQH = number of inflorescences per point-quadrat hits, RS/GS = reproductive structures per generative shoot. Direction indicates the direction of the correlation.

	R^2	p-value	direction
FGS			
L. helveticus	0.1502	0.3027	+
P. aurea	0.1474	0.3077	+
R. villarsii	0.0423	0.5956	-
NI/PQH			
B. media	0.0094	0.8039	+
C. sempervirens	0.1610	0.2844	+
F. violacea	0.0223	0.7011	+
H. versicolor	0.1074	0.3893	+
N. stricta	0.0260	0.6783	+
RS/GS			
P. aurea	0.0042	0.8681	-

3.4 Effects on flowering phenology

Flowering phenology of two (out of three) analysed species was significantly influenced by nitrogen deposition. The fraction of flowering generative shoots (FFGS) of *P. aurea* was significantly reduced with elevated nitrogen deposition at all three measuring dates (Table 7, Fig. 3), but delay of flowering was only minor. A maximal delay of two days was recorded at FFGS = 0.5 (half of the generative shoots reached the phenological stage “flowering”) in the N50 treatment compared to the N0 treatment. The FFGS of *R. villarsii* showed a significant non-linear ozone x nitrogen interaction (Table 7, Fig. 3). In the AA and 2 x AA ozone treatments, flowers were developed earlier in the N50 treatment compared to the other treatments, but in the 1.5 x AA ozone treatment N50 showed a delayed development. The FFGS of *L. helveticus* showed no reaction to the ozone or nitrogen treatment, but time of first flowering seemed to be slightly, but not significantly, positively influenced by warmer temperatures (Table 6). A weak, not significant positive correlation with temperature was also recorded for the first measuring date of the FFGS of *R. villarsii*. No temperature effect was recorded for *P. aurea.* Throughout the growing season, greenness of canopy (NDVI) showed no significant correlation with temperature.

Table 6. Mean phenological development of flowers and mean canopy greenness (NDVI) correlated with mean temperature of fumigation rings. FFGS = Fraction of flowering generative shoots; Flowers/RS = Number of flowers per totally reproductive structures present; NDVI = normalized difference vegetation index; direction indicates the direction of correlation.

	Date	R^2	p-value	direction
FFGS				
L. helveticus	23.06.	0.2899	0.1347	+
	01.07.	0.0749	0.4761	+
	06.07.	0.0057	0.8473	-
P. aurea	07.06.	0.0033	0.8827	-
	16.06.	0.0358	0.6257	+
	23.06.	0.0978	0.4126	+
R. villarsii	27.05.	0.2951	0.1306	+
	07.06.	0.1085	0.3868	+
	16.06.	0.0078	0.8209	-
Flowers/RS				
P. aurea	07.06.	0.0121	0.7779	+
	16.06.	0.0149	0.7541	-
	23.06.	0.0323	0.6435	-
NDVI	03.05.	0.0614	0.5203	-
	06.06.	0.2320	0.1892	+
	26.06.	0.0071	0.8292	+
	10.07.	0.1493	0.3043	-

Table 7. Results of repeated measures split-plot ANOVA of flowering phenology; FFGS = fraction of flowering generative shoots, D = day, RS = reproductive structures, (*) p<0.1, *p<0.05, **p<0.01, *** p<0.001, ns = not significant (factor excluded during model reduction), X = factors not tested, (day x day interactions are not possible with 2 measurements).

FFGS	Block	O_3	D	DxD	N	NxN	O_3xN	O_3xNxN	O_3xD	O_3xDxD	NxD	NxDxD	O_3xNxD
L. helveticus	0.9057	0.6133	<0.0000***	<0.0001***	0.1622	ns	ns	ns	ns	ns	ns	ns	ns
P. aurea	0.5445	0.5117	<0.0001***	0.0143	ns	0.0416*	ns	0.0281*	ns	ns	ns	ns	ns
R. villarsii	0.2104	0.8910	<0.0001***	<0.0001***	0.0239*	ns	ns	ns	ns	ns	0.1890	ns	0.0706(*)
Flowers/RS													
P. aurea	0.5596	0.8697	<0.0001***	X	0.7805	ns	ns	ns	ns	X	ns	X	ns

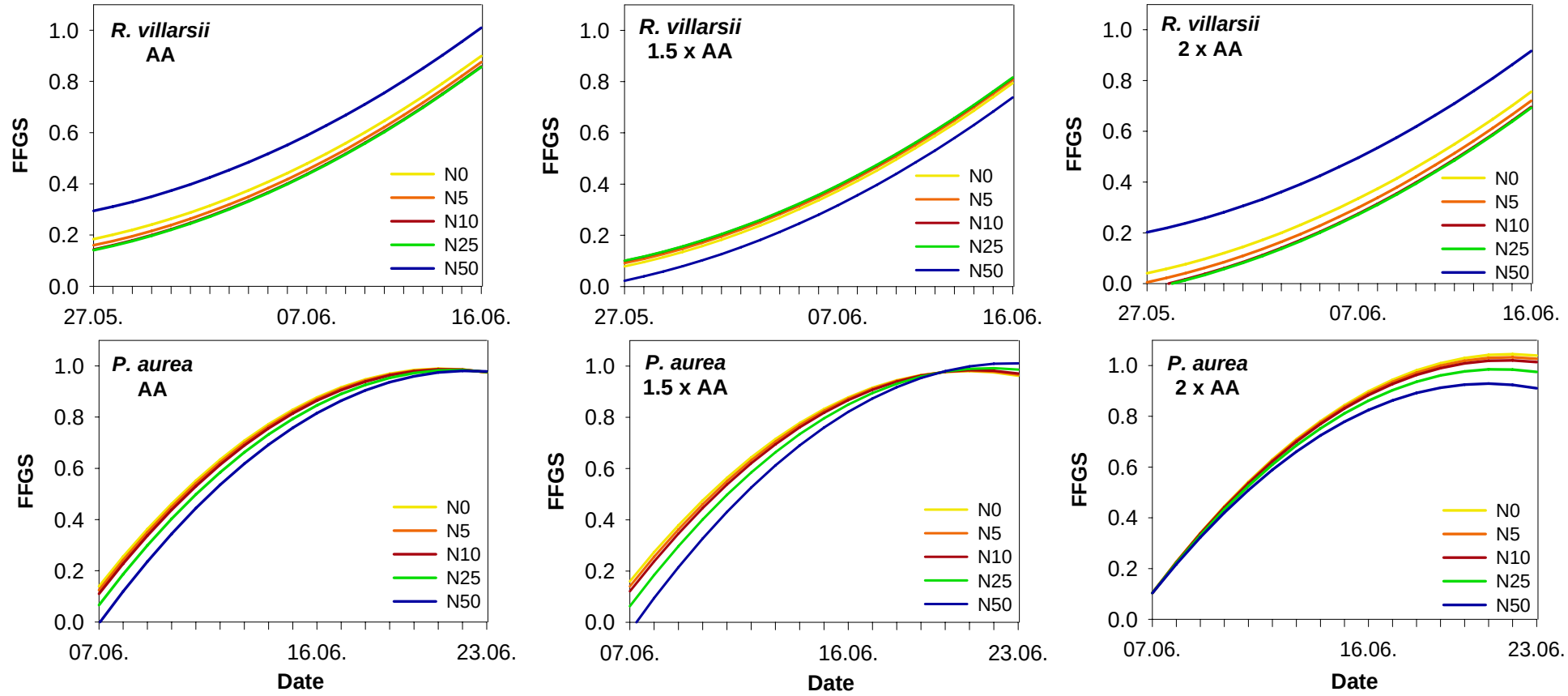

Fig. 3: Effects of ozone and nitrogen on the fraction of flowering generative shoots (FFGS) of *P. aurea* and *R. villarsii*, modelled data. AA = ambient air ozone.

4 Discussion

4.1 Effects of ozone

In the current experiment, most of the 12 investigated species were not affected by ozone in terms of abundance, flower production or flowering phenology. The only, but quite strong, observed significant ozone effect was a decreased flower production of *C. sempervirens*. This species showed only half the number of inflorescences per point-quadrat hits (NI/PQH) in the elevated ozone treatments compared to the AA treatment. Another graminoid species, *H. versicolor*, showed a reduced flower production, but the effect was only marginally significant.

Very similar results were obtained by Bergmann, Bender, and Weigel (1996), who studied 17 herbaceous (low-land) species and did not find an ozone effect on time of flowering, but two species showed a reduced number of inflorescences. However, in their study two species produced more inflorescences with elevated ozone. Experiments carried out with graminoids both showed negative (Taylor et al., 2002) and no ozone effect on flower production (Danielsson, Gelang & Pleijel, 1999). Therefore it cannot be assumed that graminoids are more susceptible to ozone in terms of flower production.

It is not clear if projected future ozone concentrations do not affect flower production of most of the investigated alpine plants, or if the reactions will take place on a longer time scale. Another open question is, to what extent the allocation of resources to roots, vegetative, and generative parts of the plant is genetically fixed at species level. In other words, can plants react to an environmental stress by reducing their investment in sexual reproduction? For alpine plant species, faced with many environmental stresses and commonly showing different reproductive strategies, the answer might be yes.

The recorded reduction of flower production in *C. sempervirens* and *H. versicolor* may be a result of direct ozone effects on reproductive structures (as reported by Bosac et al., 1994) or indirect effects on photosynthetic structures, leading to lack of resources. Bergmann, Bender, and Weigel (1996) observed a relation between extent of leaf injury and reduction of reproductive organs and suggested that indirect effects were responsible for reducing the number of reproductive organs of the species they had investigated. Field observations at Alp Flix and analysis of chlorophyll content (Bassin, unpublished) indicate that leaves of *C. sempervirens* had larger senesced parts in the 2 x AA treatment compared to the AA treatment. Therefore reductions of flower production of *C. sempervirens* might be a result of negative impacts of ozone on leaves. Such an alternation in resource allocation seems plausible as *C. sempervirens* is mainly reproducing clonally.

The study was carried out in the third year of the experiment and therefore it might be too early to see abundant effects of ozone on these slow-growing, stress-resistant plants. A similar long-term free-air fumigation experiment, carried out on a mid-elevation grassland (754 m a.s.l.), supports this assumption. A significant reduction in biomass was only observed after five years of ozone fumigation (Volk et al., 2006), although this highly productive system seems to be more susceptible to environmental stresses than an alpine plant community.

4.2 Effects of nitrogen

Contrary to initial assumptions, none of the investigated species showed significantly enhanced or accelerated flower production with elevated nitrogen deposition, although *F. violacea* tended to produce more inflorescences in the intermediately fertilized plots. Flower development of *P. aurea* was even a few days retarded in the high nitrogen treatment. In terms of species abundance, *C. sempervirens* was the only species showing a strong positive reaction to nitrogen application.

These results contrast with the common assumption that nitrogen promotes flowering, as reported for many high-elevation species (*Chuquiraga oppositifolia*, (Muñoz et al., 2005), *Erica cinerea* (Leith et al., 1999), *Pulsatilla vernalis* (Kellner, 1993), *Lolium perenne* (Wagner et al., 2001), and *Calluna vulgaris* (Power et al., 1995)). However, some species did not show a nitrogen effect on flower production (*Nardus stricta*, *Eriphorum vaginatum* (Leith et al., 1999), and *Calluna vulgaris* (Calvo et al., 2005)).

It is unclear why nitrogen application retarded flowering of *P. aurea*. Cleland et al. (2006) also found a delayed flowering in grasses (2 - 6 days), but forbs showed an accelerated flowering with nitrogen fertilization (2 - 4 days). The range of the delay is about the same as in the current study and might not impair the success of sexual reproduction. However, nitrogen might additionally have a delaying effect on seed development of *P. aurea*. For a species growing at high elevation, where the snow-free period is short and mowing of grasslands additionally reduces the plants time to produce mature seeds, an accumulated delaying effect could impair success of sexual reproduction. However, seed development has not been subject of the current study and a follow-up study would be needed to address this topic.

The overall weak reaction of the investigated species to nitrogen application can be explained by the already mentioned slow reaction of alpine plant communities to new growing conditions. Another explanation is a possible co-limitation by other factors, such as phosphorous or water (Soudzilovskaia & Onipchenko, 2005; de Valpine & Harte, 2001). In that case, species cannot profit from additional nitrogen and anthropogenic nitrogen deposition would have a smaller impact on this plant community than expected. First results from a fertilization experiment carried out at Alp Flix, using single nitrogen and phosphorous, and their combination, indicate that phosphorous could be a co-limiting element (Riesen, unpublished data). Soudzilovskaia & Onipchenko (2005) suggest that *Carex* spp. is little limited by phosphorous, having cluster roots that increase the capacity of phosphorous scavenging and uptake. This could explain why only *C. sempervirens* strongly increased its vegetative growth with additional nitrogen, while other species were likely more co-limited by phosphorous.

It is interesting to note that *C. sempervirens* apparently profited from additional nitrogen, but did not invest more in its reproductive structures. For this mainly clonally propagating species additional allocations to generative structures might not be an advantage in a densely inhabited plant community, where establishing of new seedlings is difficult. Additionally, many shoots growing in the elevated nitrogen treatments were possibly still too young to reproduce sexually, since they can only produce inflorescences in the second year (Schroeter, 1926). Comparison of point-quadrat data from the year 2004 (onset of the experiment) and 2006 revealed a significant increase of PQH with elevated nitrogen

deposition (Bassin, unpublished data). Therefore, the fraction of young shoots is likely to be higher in the elevated nitrogen treatments and the fraction of shoots able to produce flowers is lower. However, a simultaneous increase in number of plants and generative shoots of *Carex* spp. (consisting of *C. sempervirens, C. caryophyllea*, and *C. umbrosa*) with nitrogen fertilization was found by Soudzilovskaia & Onipchenko, (2005). In the same study, *Helictotrichon versicolor* showed a decreased number of plants and generative shoots with nitrogen fertilization, whereas nitrogen did not affect this species in the current study. The contrasting results can be explained by the nearly two-fold nitrogen input applied (90 kg N ha^{-1} yr^{-1} over 5 years compared to maximal 50 kg N ha^{-1} yr^{-1} over 3 years in the current study). Additionally, the species composition under investigation plays an important role for the outcome of an experiment. Most species would react positively to nitrogen application in a nutrient-poor environment if grown alone. Under competition, reactions to fertilization may be different and the species profiting most from the additional nitrogen may out-compete others.

4.3 Ozone x nitrogen interaction

Three significant ozone x nitrogen interactions were recorded, but the interactions were contrasting and not easily interpreted. *Ranunculus villarsii* showed a significant ozone x nitrogen interaction on its flower production and flowering phenology and *N. stricta* showed an ozone x nitrogen interaction on its abundance. Two other species (*L. mutellina* and *P. aurea*) showed a significant ozone x nitrogen effect on their abundance, but the effect was just an artefact of the species distribution by onset of the experiment, (see section 4.5).

Presently, two possibilities of ozone x nitrogen interactions are being discussed. First, increased nitrogen supply could increase plants sensitivity to ozone due to stimulated growth and higher specific leaf area (SLA). Alternatively, nitrogen could increase ozone tolerance due to enhanced detoxification capacity through stimulated photosynthesis (Bassin, Volk & Fuhrer, 2006). The findings of this study do not clearly support one or the other interaction-type, since the 1.5 x AA and 2 x AA treatment mostly showed opposing effect. It is interesting that no significant ozone x nitrogen interactions were recorded for *C. sempervirens*, although this species strongly reacted to ozone and nitrogen. To understand the complex interactions of ozone and nitrogen on semi-natural vegetation, further research is needed.

4.4 Effects of microclimate

Ozone had less effect on the investigated species than initially assumed, but this is not due to superposing microclimatic site effects. Although microclimatic variability is quite large among plots, temperature did not correlate with flower production or flowering phenology of most species. Also, canopy development did not show a significant correlation with temperature. A slight positive correlation may have existed at June 6, 2006, but was not strong or significant.

The flower development of *L. helveticus* showed a weak positive correlation with temperature at time of first flowering (June 23), whereby ring 1 was an outlier. Excluding ring 1 resulted in a strong and significant correlation (R^2 = 0.6513; p = 0.0155). However, no plausible

explanation could be found as to why ring 1 should show a different flower development and would justify such an exclusion from the analysis. Temperature may have had a side effect on flower development of *R. villarsii*. In the 1.5 x AA treatment, which showed below average temperatures, flower development was retarded with increasing nitrogen deposition, whereas it was accelerated in the AA and 2 x AA treatment. However, there is little evidence for this assumption, as no significant correlation of temperature and flowering phenology was found for this species.

The rearrangement of monoliths at the end of every growing season may have helped to minimize microclimatic site effects. Carry-over effects are important for alpine plants, which have been shown to preform buds in the previous or even pre-previous season (Körner, 1999). Preformation of buds is most commonly found in early-flowering species allowing them to flower right after snow-melt. The investigated species all flower in mid- or late-season and preformation of buds may be less important. Correlations of the temperature of last years fumigation ring did not show a positive interaction between last year's temperature and this year's flower production. The findings of this study are supported by Totland and Alatalo (2002), who suggested that date of snowmelt has an overwhelming effect on phenology of *Ranunculus glacialis* and that growing season temperature has little influence on speed of phenological development. Since date of snowmelt was standardized, microclimatic variability may not have had a large influence on flowering of the investigated species.

4.5 Experimental limitations

Effects of air pollutants on (semi-)natural vegetation need to be studied under realistic experimental conditions. Established plant communities grown under natural conditions are likely to react differently to environmental changes than single plants grown in pots in artificial soil (Bassin, Volk & Fuhrer, 2006). The disadvantage of realistic experimental conditions is the broad temporal and spatial variability and possible confounding effects, eventually bringing about misleading results. Data obtained in a single season, as was the case in this study, are therefore not very reliable since interannual variability can be large, as reported in most comparable studies (Rämö et al., 2006; Muñoz et al., 2005; Leith et al., 1999). The 2006 growing season was very dry, resulting in a possible co-limitation by water, which would reduce nitrogen effects on flower production and phenology. Repeating observations in following growing seasons would reveal if the effects are replicable and consistent among years. Due to heterogeneity of the investigated grassland, a very large replication of treatments would be needed to provide unbiased samples. In practice, this is not feasible. Therefore, artefacts can result from uneven starting conditions. The analysis of species abundance gives a good example for this circumstance:

The abundance of *G. acaulis*, *T. alpinum*, and *A. montana* showed nitrogen-dependent patterns. The comparison of point-quadrat data of the first year of the experiment with this year's data revealed that *G. acaulis* showed a similar distribution pattern already at onset of the experiment, and the observed distribution is therefore an artefact. In contrary, the only marginally significant ($p = 0.07$) negative nitrogen effect on the abundance of *A. montana* in 2006 turned out to be the result of a significant ($p = 0.0058$) non-linear nitrogen effect over the years. The number of PQH decreased in all treatments, but was the strongest in the N50

treatment, with only half as many PQH in 2006 compared to 2004. On the other hand, the lower abundance of *T. alpinum* in the high nitrogen treatments was not an effect of nitrogen application. In the N0 to N25 treatments, the species' abundance remained nearly unchanged over the years, but in the N50 treatment a significant *increase* was recorded in the AA ozone treatment (highly significant ozone x nitrogen interaction; $p < 0.0001$). Looking at those examples, the importance of long-term observations and the danger of drawing conclusions from single year data become evident.

The observed phenological changes were smaller than initially expected. Therefore, the time interval between measurements of about one week was too large for the scale of changes of only a few days. Thus, reliability of these results is limited. The current study focused on flower production and time of flowering, two important parameters for success of sexual reproduction. Nevertheless, ecological relevance of observed effects can only be determined by investigating the success of sexual reproduction as a whole, thus flower and seed production, abortion rates, and seedling establishment.

4.6 Conclusions

The recorded effects of ozone and nitrogen on flower production and phenology were smaller than expected and no directed ozone x nitrogen interaction was found. It remains unclear, if three years of simulated environmental changes were too short for detecting strong effects or if the investigated stress-tolerant alpine species are not sensitive to projected future concentrations of ozone and nitrogen in terms of flower production and flowering phenology.

In the future, alpine species-rich pastures will probably not only face higher atmospheric nitrogen inputs and higher ozone concentrations, but also elevated CO_2 concentration and a changing climate. Additionally, due to increasing skiing and hiking tourism and ongoing abandonment of alpine farms, alternations in land-use patterns are likely. If such drastic environmental changes occur, species either have to adapt to new growing conditions or migrate to a site that better fits their traditional habitat. Sexual reproduction plays a key role in both processes. Nevertheless, interactions of anthropogenic environmental changes on sexual reproduction are complex and poorly understood. Future research should focus on overall success of sexual reproduction, considering abortion-rates of buds, flowers, and seeds, germination rates, and seedling establishment.

Even if there is still a wide gap of knowledge concerning the effects of global change, precautionary measures should be taken. To conserve alpine biodiversity, sustainable land-use concepts have to be developed and combustion of fossil fuels has to be drastically reduced, since this is not only a major source of air pollutants but also of greenhouse gases, causing climate change. At our experimental site, a first step towards sustainable land-use has been taken in 2006, when Parc Ela, a natural park covering two valleys, 21 communities, and an area of 600 km^2, was established, with the goal of combining nature conservation and economic development.

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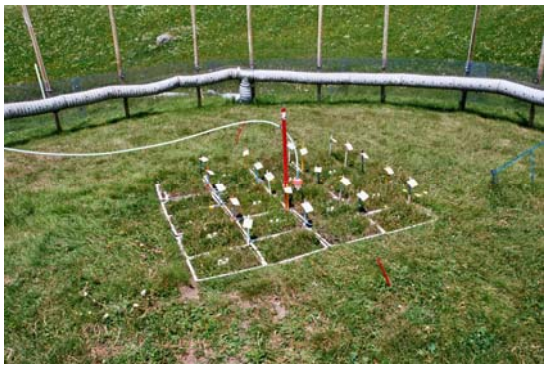
7 Appendix

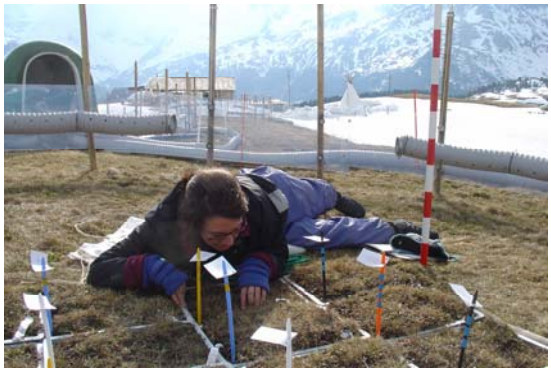
experimental site

NDVI measuring



fumigation ring

turf monolith



author during fieldwork I

author during field work II

Potentilla aurea shoots

marked *Potentilla aurea* flowers

Gentiana acaulis flower

Leontodon helveticus with pollinator

Ligusticum mutellina

Trifolium alpinum

Nardus stricta and *Helictotrichon versicolor*

Carex sempervirens