The influences of N_2-fixing *Alnus* and atmospheric nitrogen deposition on the nitrogen balance in close to natural ecosystems

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Chapter 1

General introduction

Nitrogen (N) is a key plant nutrient and is needed for a large number of organic compounds, e.g., nucleic acids, amino acids, proteins, enzymes, and adenosine triphosphate molecules. Nitrogen is driving many key metabolic processes involved in growth but also energy transfer reactions. The largest potential source of N represents the atmosphere with molecular N_2 as the most abundant gas (78%). However, the triple bond in the molecular N_2 is one of the strongest, i.e., N_2 needs to be either reduced, for example to ammonia (NH_3), or oxidised to nitrate (NO_3^-) to form reactive N (named N_r hereafter), thus to become available for all non-N_2-fixing organisms (Schulze et al., 2002). Since bedrock contains almost no N, external N inputs are needed to build up a biologically available N pool in terrestrial ecosystems. Although soils may contain large amounts of N, it is often tightly tied to soil organic matter and therefore not readily plant available. In close to natural ecosystems, the N cycle is largely closed and internal N recycling covers most of the N demand of plants and microbes. In these ecosystems, N losses through leaching in the form of NO_3^- or dissolved organic N (DON), and gaseous emissions from denitrification (nitric oxide, NO; nitrous oxide, N_2O; molecular N, N_2) or litter export are generally small. These minor N losses are commonly offset by N_2 fixation and/or natural atmospheric N deposition. Molecular nitrogen is either transformed to ammonium (NH_4^+) by free-living bacteria such as cyanobacteria or by bacteria living in symbioses with plants or fungi (e.g., cyanolichens). Well known examples of N_2-fixing symbioses are bacteria in root nodules of plants such as *Acacia* or *Trifolium* species living in symbiosis with rhizobia, or *Alnus* and *Hippophae* species that are associated with *Frankia.* Symbiotic and non-symbiotic N_2 fixation are generally in the range of 2.3–3.1 kg N ha^{-1} a^{-1} in natural grassland and 6.5–26.6 kg N ha^{-1} a^{-1} in forests, in cases where N_2-fixing plants share between 1% and 10% of the total cover of trees and the understorey (Cleveland et al., 1999). Pre-industrial, atmospheric N deposition was most likely below 2 kg N ha^{-1} a^{-1} in Europe (Butterbach-Bahl et al., 2011) and in the absence of human activities, atmospheric background N deposition from lightning and natural fires was estimated to be below 0.5 kg N ha^{-1} a^{-1} (Galloway et al., 2008).

Since the industrial revolution, human activities have dramatically and globally increased the amount of N_r. The total annual production of N_r amounted to c. 15 Tg N a^{-1} in pre-industrial times (1860), amplified to 156 Tg N a^{-1} in 1995 and to 187 Tg N a^{-1} in 2005 and is further predicted to increase worldwide (Galloway et al., 2008). Europe has been one of the biggest producers of N_r, nevertheless and thanks to abatement strategies N emissions have been decreasing since the late 90s (mainly nitrogen oxides, NO_x; Erisman, 2011). Yet, the continuous demand for nitrogen in agriculture has steadily increased the production of N_r through the Haber-Bosch process and the large-scale cultivation of N_2-fixing plants (Galloway et al., 2004; Fowler et al., 2013). Through the combustion of fossil fuels and biomass, N is mainly emitted in oxidised forms to the atmosphere (NO_x). Inefficient use of N_r, for instance through agricultural practices, causes further losses of N_r to the air (mostly as NH_3) and water (NO_3^- or DON). In the atmosphere, emitted N_r is transported over short (NH_3) or long distances (NO_x) and deposited as gas, particulate matter or wet by precipitation. Today, the total atmospheric N deposition is in the range of 5 kg N ha^{-1} a^{-1} to 60 kg N ha^{-1} a^{-1} across Europe (Kesik et al., 2005; EMEP, 2013). In Switzerland, it regularly exceeds 40 kg N ha^{-1} a^{-1} in the lowlands with intensive agriculture and is in the range of 5–15 kg N ha^{-1} a^{-1} in the Swiss Alps.

Member countries to the international conventions on greenhouse gas emissions and air pollution have to report their national emissions according to the guidelines of IPCC (2006) and EMEP/EEA (2013). Understanding the interactions between N input and thereby induced indirect N_2O and NO emissions is important to estimate national emissions of greenhouse gases and air pollutants. In agricultural systems with commonly high N loads by fertilizer, the relationship between N input and induced N_2O and NO emissions is well documented (Stehfest and Bouwman, 2006). However, it is largely unknown to which extent atmospheric N deposition induces N_2O and NO emissions in more natural ecosystems such as extensively used grassland or wetlands and forests. Hence, the total national indirect N_2O and NO emissions are quite uncertain in countries with a large share of close to natural ecosystems like Switzerland.

Over the recent decades, shrub encroachment into grasslands, savannas and open woodlands has been reported for many parts of the world, either resulting from land use changes, increased N deposition, reduced fire frequencies, climate warming or the presence of exotic plants (Eldridge et al., 2011). Also elevated CO_2 has been discussed as a potential driver, particularly in drier regions (Morgan et al., 2007). Among these expanding shrubs, there are many N_2-fixing species such as *Alnus viridis* (green alder) spreading into grasslands in the Alps, Siberia, NW-Canada and the Balkan and Carpathian mountains (Hiltbrunner et al., 2014 and citations therein), or *Myrica faya* invading young volcanic sites in Hawaii (Vitousek et al., 1987). Currently, *Alnus viridis* represents the most rapid land cover change in the Alps. Naturally, this shrub is restricted to disturbed habitats, such as moist ravines or edges of watercourses. From these habitats *Alnus* spreads into montane grassland due to the reduced use or abandonment of centuries-old meadows and pastures (Fig. 1.1). Today, *Alnus viridis* covers a total of c. 50'000 ha in Switzerland (calculated form data of Brändli 2010). This fast transformation of nutrient-poor grassland into species-poor, N-rich *Alnus* thickets threatens not only biodiversity, but also alters N fluxes and pools at a large scale. In comparison to atmospheric N deposition, symbiotically N_2-fixing plants may introduce larger loads of N_r into ecosystems, often exceeding 100 kg N ha^{-1} a^{-1} (Binkley, 1981; Cleveland et al., 1999).

Irrespective of its origin, i.e., atmospheric N deposition or N_2 fixation, large inputs of N_r induce a cascade of effects, because N_r interacts with chemical processes in the soil and exerts differential outcomes on organisms (Galloway et al., 2003). The threshold below which no 'significant harmful effects on specified sensitive elements of the environment' are expected is called critical load for nitrogen (CLN; Nilsson and Grennfelt, 1988). The CLN differs between ecosystems and is set to 5–10 kg N ha^{-1} a^{-1} for raised bogs, to 10–20 kg N ha^{-1} a^{-1} for montane hay meadows and to 10–15 kg N ha^{-1} a^{-1} for montane coniferous forests (Bobbink et al., 2011). At present, the CLN is exceeded by 30% in close to natural vegetation in western Europe and by 80% in eastern Europe (Dentener et al., 2006; CLN of 10 kg N ha^{-1} a^{-1} in that study). In case when *A. viridis* encroaches montane grassland, the CLN of these former grasslands is by far exceeded by *Alnus'* N_2 fixation. Nitrogen fixation rates by *Alnus viridis* are assumed to be in the range of 20–62 kg N ha^{-1} a^{-1} (Binkley, 1981).

When CLN is exceeded, this commonly leads to losses in biodiversity, soil acidification, and an accelerated N cycle. For instance, increased availability

Figure 1.1: A not yet invaded pasture and an *Alnus viridis* thicket in the foreground, and a slope covered with *Alnus* shrubs in the background in the Ursern valley.

of N_r causes fast-growing species to outcompete slow-growing species, resulting in a reduced plant species diversity and evenness (Bobbink et al., 2010; Payne et al., 2013). High N_r loads may not only speed up N fluxes, but also increase turnover rates of N pools (Fig. 1.2). Thus, increased N_r may augment the size of N pools (through higher net primary production and accumulation of soil organic matter), but pool size may also decrease due to amplified N losses. If the N_r load exceeds the biological demand for N and the storage capacity of soils, the ecosystem gets N saturated according to the definition by Aber et al. (1989), causing N losses. Thus in N saturated ecosystems, NO_3^- and DON are leached and N is emitted as N_2O, NO and N_2 to the atmosphere. Therefore, high loads of anthropogenic atmospheric N deposition induce substantial N_2O and NO emissions from soils (Pilegaard et al., 2006). Besides accelerating the N cycle, high N_r input interacts with other nutrients (e.g., basic cations, Fe) and with the C cycle. It is debated whether a higher N availability may increase the C sequestration in forests (Nadelhoffer et al., 1999, de Vries et al., 2009) and how it affects N stocks in close to natural ecosystems (Butterbach-Bahl et al., 2011).

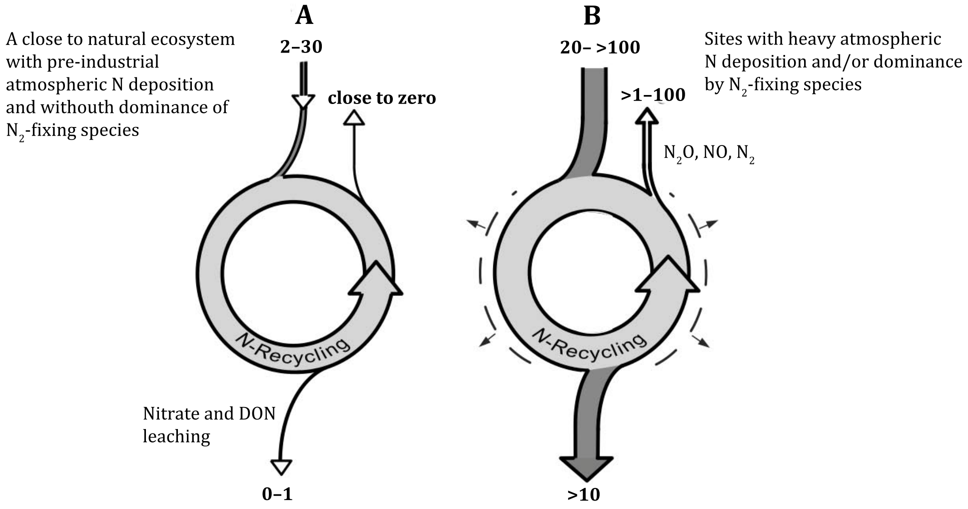

Figure 1.2: A schematic illustration of the N cycle in **A** an ecosystem with pre-industrial atmospheric N deposition and less than 10% cover of N_2-fixing species versus **B** a system with high atmospheric N deposition and/or a dominance of N_2-fixing species. Numbers are given in kg N ha^{-1} a^{-1} and represent a combination of values from the literature and field measurements (adapted from Hiltbrunner et al., 2014).

Content of this PhD thesis

This thesis had to meet the specific requirements of the Mercator Foundation Switzerland as part of a Zürich-Basel Plant Science Center (PSC) fellowship program, namely to also cover science-policy issues. This science-policy part emerged from two internships, one at the Swiss Academy of Sciences (Swiss Biodiversity Forum) and one at the Federal Office of the Environment (FOEN). The products of the internships represent an integral part of this thesis (chapter 2 and 3). Following this introductory chapter, the thesis is composed of the subsequent four chapters:

Chapter 2, provides a model for so-called indirect N_2O and NO emissions induced by atmospheric N deposition in close to natural ecosystems in Switzerland, based on literature data. The approaches employed are applicable in the Swiss Emission Inventory (EMIS), and thus, help to improve the quantification of the national indirect N_2O and NO emissions.
Published in Atmospheric Environment.

Chapter 3, explains the causes and consequences of the rapid encroachment of *Alnus viridis* into high elevation grassland for a broad audience and discusses potential management options. This publication was complemented with a 'factsheet' in German and French released to Swiss media.
Published in Alpine Botany; factsheet by SCnat.

Chapter 4, presents the results from a field study (2009–2012) on the effects of the encroachment of *Alnus viridis* into grassland on N pools and fluxes in three valleys at two elevations each in the Swiss Alps. The work included soil characteristics such as base saturation and pH, an estimation of the ecosystem N and carbon pools, and nitrate leaching. The total N concentration in the soil solution was monitored at a high temporal resolution. Stream water quality was explored for streamlets of four micro-catchments differing in *Alnus* cover (cover varied between c. 10% up to 90%).
Published in Ecosystems.

Chapter 5, reports the influence of *Alnus* driven N_2O emission rates across an elevational gradient that represents a 5.7 K temperature range. This chapter illustrates the differential influences of a warmer climate and species identity (N_2-fixing versus non-N_2-fixing) on the emission of this powerful greenhouse gas.
Published in the European Journal of Soil Science.

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Chapter 2

Induction of indirect N_2O and NO emissions by atmospheric nitrogen deposition in (semi-)natural ecosystems in Switzerland

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Abstract

During the past century atmospheric nitrogen deposition increased dramatically due to human activities worldwide. Currently, it exceeds the critical load for nitrogen (CLN) in over 90% of the Swiss forest area and raised bogs, in 80% of all fens and in 30% of species-rich grassland areas in Switzerland. Indirect gaseous nitrogen losses (HNO_2, NO, N_2O, N_2) from these soils induced by atmospheric nitrogen deposition are likely to be substantial. However, the approaches to estimate these indirect N emissions provided by the international organisations (UNFCCC, IPCC; UNECE, EMEP/EEA) are based on agricultural data only. They may not be suitable to estimate the indirect emissions from (semi-)natural ecosystems such as forests, extensively used grassland, and wetlands. The present study aims at calculating ecosystem-specific annual indirect N_2O and NO emissions of (semi-)natural ecosystems in Switzerland for the years 1990, 2000, 2007 and 2010 using a simple linear model similar to the international guidelines. The approach here is based on empirical data for (semi-)natural ecosystems, derived from a literature survey, is driven by atmospheric nitrogen deposition and is ecosystem-specific with a high spatial resoldlution of 100 m x 100 m. Our results show that such ecosystems represent a strong source of indirect N emissions induced by atmospheric nitrogen deposition and emitted 1.61 ± 0.32 Gg N_2O-N and 2.51 ± 0.53 Gg NO-N into the atmosphere in Switzerland in the year 2010, corresponding to 21% of the total Swiss N_2O emissions and 10% of the NO_x emissions. Thanks to the reduction of N emissions and thereby reduced atmospheric N deposition, the indirect N_2O and NO emissions from (semi-)natural ecosystems are estimated to have been both reduced by c. 20% from 1990 to 2010. We conclude that the source strength for N_2O and NO emissions of (semi-)natural ecosystems have been underestimated by the current approaches of IPCC and EMEP/EEA by a factor of 4.4 and 17, respectively. In regions and countries with a high fraction of (semi-)natural ecosystems, the approach offered here has the potential to improve the estimate of indirect N emissions substantially.

1. Introduction

Human activities have led to a substantial increase in nitrogen (N) emissions and atmospheric N deposition worldwide: between 1860 and 1990 N emission increased from 33.7 Tg N a^{-1} to 104.1 Tg N a^{-1} and hence, N deposition increased in the same order (Galloway et al., 2004). In the absence of human activities, N deposition would generally be lower than 0.5 kg N ha^{-1} a^{-1} (Galloway et al., 2008). Currently, it exceeds the critical load for nitrogen (CLN) in 30% and 80% of natural vegetation in Western and Eastern Europe, respectively (Dentener et al., 2006; CLN was defined as a threshold of 10 kg N ha^{-1} a^{-1} in that study). Thus, in affected ecosystems, significant harmful effects on specified sensitive elements of the environment, such as changes in plant species richness, nutrient imbalances, or increased N emissions are likely to occur (Nilsson and Grennfelt, 1988). Soils exposed to high N deposition and nitrogen-rich soils often show nitrogen saturation (Aber et al., 1989). As a consequence, N is leached as nitrate (NO_3^-) or emitted into the atmosphere as nitrous acid (HNO_2), nitric oxide (NO), nitrous oxide (N_2O) or molecular nitrogen (N_2) as products of microbial nitrification and denitrification processes (Gasche and Papen, 1999; Ludwig et al., 2001; Oswald et al., 2013; Papen and Butterbach-Bahl, 1999). Therefore, N deposition correlates positively with N_2O and NO emissions in many (semi-)natural ecosystems. We use the term (semi-)natural ecosystem for ecosystems that are used extensively only and with net input from atmospheric N deposition only. The N emissions induced by atmospheric N deposition are in the following referred to as indirect emissions. Parties to international conventions and protocols on air pollutant and greenhouse gas emission control should also report their indirect N_2O emissions to UNFCCC (IPCC, Kyoto Protocol) and indirect NO_x (nitrogen oxide, including NO) emissions to UNECE (CLRTAP, Gothenburg Protocol). N_2O is an extremely strong greenhouse gas with a high global warming potential of 298 compared to CO_2 over 100 years (Myhre et al., 2013). NO_x contributes to eutrophication and acidification of ecosystems and to the formation of tropospheric ozone (Butterbach-Bahl et al., 2011; Hertel et al., 2011).

1.1. Atmospheric nitrogen deposition in Switzerland

Over the past two decades, total atmospheric N deposition (wet and dry) decreased from 86.5 Gg N in 1990, to 73.3 Gg N in 2000 to 68.4 Gg N in 2010, of which 43% was deposited into (semi-)natural ecosystems in the year 2010 (Fig. 2.1). However, N deposition varies considerably in different regions and elevations of Switzerland. On the one hand, N deposition ranges from 5 to 10 kg N ha^{-1} a^{-1} for alpine and 10–15 kg N ha^{-1} a^{-1} for montane elevations in Switzerland (data from Hiltbrunner et al., 2005; model results from this study). On the other hand, N deposition frequently exceeds 40 kg N ha^{-1} a^{-1} in the Swiss lowlands due to high NH_3 emissions of intensive agriculture and NO_x emissions from combustion of fossil fuels and biomass. In the most southern part of Switzerland (the canton of Ticino), the import of N pollutants from Northern Italy contributes significantly to N deposition (FCAH, 2005). In Switzerland, N inputs by atmospheric deposition exceeded the CLN in over 90% of forest areas and raised bogs, in 80% of fens and in 30% of species-rich grassland areas (e.g., Xero- and Mesobrometum) in the year 2010 (FOEN, personal communication Beat Rihm). Therefore, the induced indirect N emissions of these ecosystems are assumed to be substantial.

1.2. Calculation of indirect nitrogen emissions from soils according to international guidelines

The Guidelines for National Greenhouse Gas Inventories 2006 by IPCC provide an approach to estimate indirect N_2O emissions from soils induced by human activities (IPCC, 2006). There, it is supposed that 1% (= emission factor EF_4) of the total NO_x-N and NH_3-N emissions of a country is re-emitted as indirect N_2O-N emission. However, this approach is neither ecosystem-specific nor linked to atmospheric N deposition. The emission factor EF_4 was set identical by IPCC to the emission factor for direct N_2O emissions of managed soils (EF_1 = 1%), because a substantial fraction of indirect N_2O emissions is thought to originate from managed land. However, this assumption is not fulfilled since 43% of N deposition is deposited in (semi-)natural ecosystems in Switzerland. Two approaches for the calculation of indirect NO-N emissions are proposed in the Emission Inventory Guidebook 2013 (EMEP/EEA, 2013). First, the

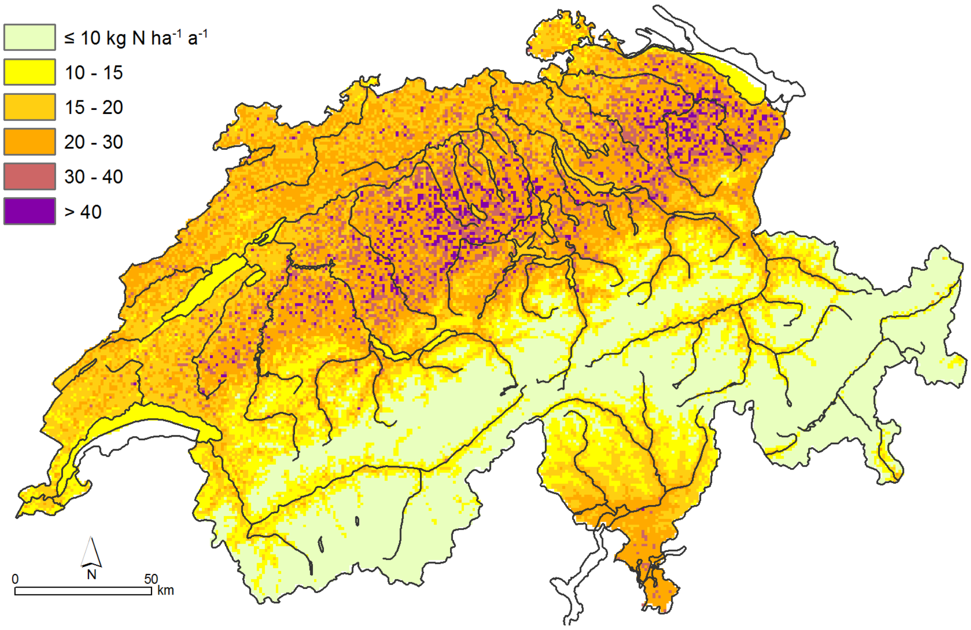

Figure 2.1: Total nitrogen deposition (wet and dry in kg N ha^{-1} a^{-1}) per km^2 for Switzerland in the year 2010.

simpler methodology of Skiba et al. (1997) is based on emission data mainly from agricultural systems. In this approach 0.3% of applied N is assumed to be returned to the atmosphere as NO-N in addition to a background emission of 0.032 kg NO-N ha^{-1} a^{-1}. By the second, detailed methodology of Novak and Pierce (1993), hourly NO emission rates are estimated that depend on land use type and on soil temperature. However, the rate of N input, which is the major driver of N emissions, is not accounted for. The two methodologies yield different annual NO emissions and are considered as uncertain (by a factor of 5; see EMEP/EEA, 2013). In contrast to indirect N emissions, the methods for modelling fertilizer-induced N_2O and NO emissions of agricultural soils by IPCC and EMEP/EEA are derived from a large dataset, and the relationships between N input and N emissions are well known (Stehfest and Bouwman, 2006). These input-output relations are less studied in close to natural ecosystems. For instance, Denier van der Gon and Bleeker (2005) highlighted that the IPCC emission factor EF_4 for the calculation of indirect N_2O emissions is underestimated by a factor of 2 and Papen and Butterbach-Bahl (1999) stated that it underrates these emissions of temperate and boreal forests significantly. According to Machefert et al. (2002) and Denier van der Gon and Bleeker (2005), these indirect N emissions may be predicted more reliably when nitrogen dynamics of soils, land use and climate are taken into account. The present study aims at providing a simple approach based on current literature in order to calculate the indirect N_2O and NO emissions of the three most relevant Swiss (semi-)natural ecosystems: forests, (semi-)natural grassland (hereafter addressed as grassland) and wetlands. Our model incorporates (1) atmospheric N deposition as the main driver of indirect N emissions (Ludwig et al., 2001; Machefert et al., 2002; Skiba et al., 2012), and (2) ecosystem-specific emission factors to calculate and to map indirect N_2O and NO emissions at a 100 m x 100 m resolution. To implement this approach in the calculation of national emission inventories, it should be comparable to the IPCC and EMEP/EEA approaches.

2. Methods

2.1. Literature survey on N_2O and NO emissions

Literature was explored for studies reporting N_2O and/or NO emissions of forests, grassland and wetlands in the temperate and boreal zones, as well as modelled or measured data of total N deposition in the corresponding ecosystem. Agricultural systems were not considered. Studies published till 2013, 1st August were included in the analysis. Since N emissions of soils are highly variable in space and time, the measurements needed to be replicated and conducted for at least one growing season (Kesik et al., 2005). We excluded any works, where fertilizer had been applied, since emissions of N compounds can be greatly amplified by fertilization, though they may be initially low, they may increase over the following years (Gundersen et al., 1998; Matson et al., 2002). Additionally, study sites with symbiotic N-fixers, such as *Alnus* or *Robinia*, were left out, since these species introduce mostly unknown amounts of N into the ecosystems. Hence, the N-sources we refer to, reflect in large consequences of atmospheric N deposition. All deposition and indirect emission data were expressed in kg N ha^{-1} a^{-1}.

For European studies, where the total N deposition was neither measured nor modelled, we referred to the dataset of EMEP (EMEP, 2011), available in a 50 x 50 km grid for the years 1980–2011, and for Swiss sites we employed modelled N deposition data from the present study (see 2.3). For several studies conducted in coniferous forests, N in throughfall was taken as a surrogate for total N deposition, although it is known that throughfall data underestimate total N deposition (Thimonier et al., 2005). To reduce this underestimation, we applied a factor of 1.2 to convert N in throughfall to total N deposition in coniferous forests (see data set of Thimonier et al., 2005).

2.2. Areal extent of the Swiss (semi-)natural ecosystems

We used the geo-referenced dataset of the Swiss Land Use Statistics (SFSO, 2007) to define the spatial area and location of forests (10,730 km^2), grassland (4,890 km^2) and wetlands (80 km^2) at a 1-ha resolution. Since these land cover types were not largely transformed into cropland or urban area, the sum of forest, wetland and grassland area has remained rather constant for the last two decades. In Switzerland, these three ecosystems receive no net N input other than atmospheric N deposition. This grassland is mostly located in montane and subalpine summer grazing areas, where it is prohibited by law to apply any fertilizer, except for manure produced in the area itself (Swiss Confederation, 2007). There may be some re-allocation of manure at local scale, but the effect on the net N budget for the ecosystem is assumed to be zero. Similarly, fertilizer applications are prohibited in Swiss forests, fens and bogs (Swiss Confederation, 2005). The Land Use Statistics enclose the whole surface of Switzerland and divide the terrain into 74 land use and land cover categories.

(1) Forests were defined using the dataset 'proportion of conifers' (SFSO, 2004), which we linked to the Swiss Land Use Statistics and divides forests into four subcategories according to the percentage of coniferous trees: (1) 91–100%, (2) 51–90%, (3) 11–50% and (4) 0–10% (SFSO, 2004). For our calculations, we took the means of each category.

(2) Grassland includes the following seven categories (codes by SFSO, 2007): 'alpine pastures and meadows' (85), 'abandoned montane grassland overgrown by shrub' (86), 'sheep pastures and alpine hay meadows' (87), 'productive montane grassland' (88), 'stony grassland' (89) and 'unproductive (*sensu* very high elevation) vegetation' (97). The category 'productive pastures' is partly fertilized. For the differentiation between most-likely-fertilized *versus* non-fertilized within this category, we left out grassland closer than 400 m to a road. The remaining grassland was assumed to be less accessible and thus non-fertilized (FOEN, 2009).

(3) Wetlands were defined by the category 'code 95' consisting of fens and raised bogs.

2.3. Swiss nitrogen deposition

Atmospheric N deposition for Switzerland was estimated and mapped on a hectare-grid for the years 1990, 2000, 2007 and 2010 using a pragmatic approach as described by Thimonier et al. (2005) and Roth et al. (2013). It combines monitoring data, spatial interpolation methods, emission inventories, statistical dispersion models and inferential deposition models. We included: wet deposition of nitrate (NO_3^-) and ammonium (NH_4^+), gaseous ammonia (NH_3), nitrogen dioxide (NO_2) and nitric acid (HNO_3) and dry deposition of particulate NH_4^+ and NO_3^- (secondary aerosols). For forests, the N deposition data were calculated using specific deposition velocities reported for coniferous and deciduous forests. These N deposition values per hectare of forest were weighted according to the fraction of deciduous *versus* coniferous trees.

2.4. Statistical analysis and calculation of indirect nitrogen emissions

Correlations between either N_2O or NO emissions (dependent variables) with atmospheric N deposition were analysed separately for coniferous forests, deciduous forests, grassland and wetlands using linear regression forced through the origin. Linear relationships between indirect N emission and N deposition in (semi-)natural ecosystems have been demonstrated by several authors (Gasche and Papen, 2002; Lohila et al., 2010; Papen and Butterbach-Bahl, 1999; Pilegaard et al., 2006; Skiba et al., 2012). As reviewed in Gasche and Papen (2002), pristine forests receiving close to zero ($<$2 kg N ha^{-1} a^{-1}) atmospheric N deposition are a very weak source of N_2O and NO emissions due to the strong competition between soil microbes and tree roots for available N. Carter et al. (2012) have shown that N_2O production is limited by N availability in nutrient poor ecosystems. In areas with very low N deposition forest soils may even function as sinks for N_2O and NO (Gasche and Papen, 2002). Furthermore, background NO-N emissions were estimated to be as little as 0.032 kg NO-N ha^{-1} a^{-1} in close to natural grassland and forests (EMEP/EEA, 2013). Therefore, we assume that at close to zero N deposition, indirect N emissions are almost zero and that background N emissions are below 0.1 kg ha^{-1} a^{-1} and do not matter for the total emissions of (semi-)natural ecosystems. This assumption is in line with the 'Emission Inventory Guidebook 2013' (EMEP/EEA, 2013) and the 'Guidelines for National Greenhouse Gas Inventories 2006' (IPCC, 2006), where emission factors (linear models forced through the origin) are applied to calculate emissions from soils.

In order to calculate ecosystem-specific emission factors and its variance, non-parametric bootstrapping with re-sampling (10,000 runs) were performed on the slopes of the linear models for coniferous forests, deciduous forests, grassland and wetlands. The resulting mean and its standard deviation (SD) of the estimated 10,000 slopes represent the mean emission factor and its SD. Subsequently, N deposition of all hectares of each land cover type was summed up. To calculate the indirect N_2O or NO emissions and its uncertainty (SD), the ecosystem-specific mean emission factor and its SD were multiplied with the corresponding total N deposition.

For grassland and wetlands, no appropriate NO emission data were available, thus we applied the 'simpler methodology' of Skiba et al. (1997) described in the 'Emission Inventory Guidebook' (EMEP/EEA, 2013). This method results in very low indirect NO emissions with a negligible uncertainty (uncertainty was not included in the analysis). We estimated the indirect N_2O and NO emissions of (semi-)natural ecosystems for the years 1990, 2000, 2007 and 2010 using the statistical package R (R Development Core Team, 2013, version 3.0.2).

3. Results

In total, 57 data sets for N_2O emission in (semi-)natural ecosystems were found that meet our criteria (Table S2.1). Most of these studies were conducted in coniferous (n = 24) or deciduous forests (n = 17) in Central and Northern Europe within an N deposition range of 0.1–47 kg N ha^{-1} a^{-1}. This covers the range of N deposition rates deposited into Swiss forests. For grassland (n = 7) and wetlands (n = 9) less data were available. In these ecosystems, N deposition varied from 1.4 to 15.0 kg N ha^{-1} a^{-1} well reflecting the range known for grassland and the lower range for wetlands in Switzerland. For NO emissions, we found 16 long-term data sets in coniferous (n = 11) and deciduous (n = 5) forests with an N deposition range of 0.7–38 kg N ha^{-1} a^{-1}. However, no suitable data on NO emissions in grassland or wetlands were found.

Table 2.1: Emission factors (mean ± SD) for indirect N_2O (N_2O-N x 1/kg N deposited) and NO-N emissions (NO-N x 1/kg N deposited) induced by atmospheric N deposition in (semi-)natural ecosystems.

Land use	EF N_2O-N	EF NO-N
Deciduous forests	0.085 ± 0.027	0.052 ± 0.021[2]
Coniferous forests	0.038 ± 0.013	0.122 ± 0.030
(Semi-)natural grassland	0.053 ± 0.008	n.a.
Wetland	0.022 ± 0.017[1]	n.a.

n.a.: Not available, instead we used the approach of Skiba et al. (1997)

[1] Not significant based on the regression calculation (see Fig. 2.2)

[2] Marginally significant (see Fig. 2.4)

3.1. N_2O emission

N_2O emission correlated statistically significant and positive with N deposition in coniferous and deciduous forests and in grassland, but not in wetlands, because too few data were available and the variance was very high (Fig. 2.2). The emission factor estimated with the non-parametric bootstrapping method was 2.2-fold higher for deciduous forests compared to coniferous forests, with the emission factor for grassland in between (Table 2.1). Although the relationship of N deposition and N_2O emission was not significant in wetlands, the data were used to at least roughly approximate the indirect N_2O emissions (Table 2.1; wetlands represent 0.5% of the total area of (semi-)natural ecosystems in Switzerland only). Annual mean air temperature and annual precipitation did not have any significant influence on N_2O emissions in the different ecosystems.

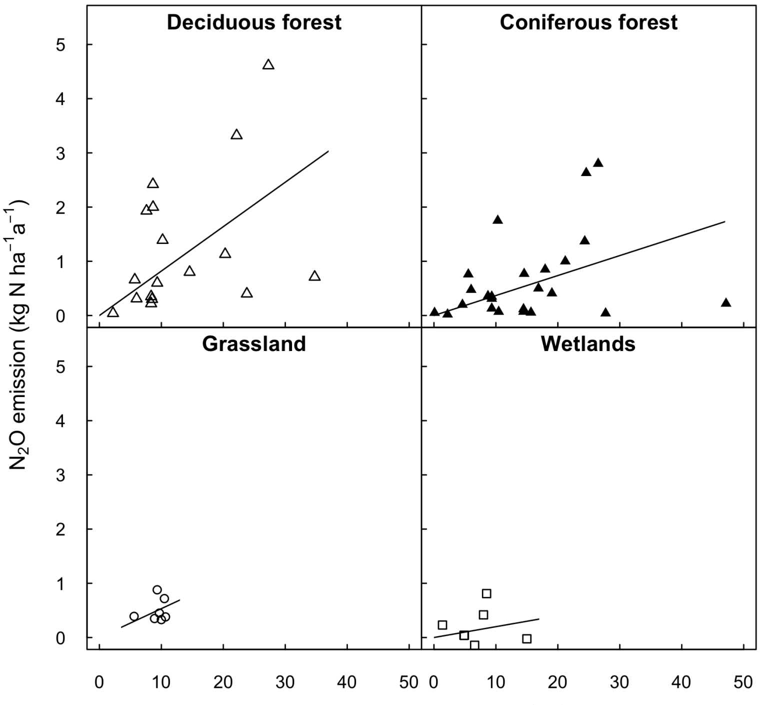

Figure 2.2: Indirect N_2O emissions in relation to total N deposition in (semi-)natural ecosystems. N_2O emissions and N deposition expressed in kg N ha^{-1} a^{-1}. Linear regressions were statistically significant in deciduous forests (n = 17, $R^2_{adj} = 0.53$, $p < 0.001$), coniferous forests (n = 24, $R^2_{adj} = 0.42$, $p < 0.001$) and grassland (n = 7, $R^2_{adj} = 0.84$, $p < 0.001$), but not in wetland (n = 9, $R^2_{adj} = 0.12$, p = 0.17).

In Switzerland, indirect N_2O emissions of (semi-)natural ecosystems were highest (>2 kg N_2O-N ha^{-1} a^{-1}) in areas with high atmospheric N deposition, particularly, in the lowlands and in the canton of Ticino, but much lower (<0.5 kg N_2O-N ha^{-1} a^{-1}) in remote, mountainous areas due to lower N deposition (Fig. 2.3). According to our estimate for the year 1990 (semi-)natural ecosystems re-emitted in total 2.01 ± 0.40 Gg N_2O-N, for 2000 1.69 ± 0.34 Gg N_2O-N, for 2007 1.62 ± 0.32 Gg N_2O-N and for 2010 1.61 ± 0.32 Gg N_2O-N in Switzerland. For the year 2010, forests re-emitted around 86%, grassland 14% and wetlands less than 0.5% (Table 2.2).

Table 2.2: Total indirect N_2O-N and NO-N emissions of (semi-)natural ecosystems in Switzerland for the years 1990, 2000, 2007 and 2010 (all in Gg; mean ± SD).

Year	Forest		Grassland		Wetland	
	N_2O-N (Gg)	NO-N (Gg)	N_2O-N (Gg)	NO-N (Gg)	N_2O-N (Gg)	NO-N (Gg)
1990	1.71 ± 0.40	3.06 ± 0.64	0.29 ± 0.05	0.03 ± 0.04	0.004 ± 0.003	0.001 ± 0.001
2000	1.45 ± 0.34	2.58 ± 0.55	0.24 ± 0.04	0.03 ± 0.04	0.004 ± 0.003	0.001 ± 0.001
2007	1.39 ± 0.32	2.50 ± 0.53	0.22 ± 0.03	0.03 ± 0.03	0.004 ± 0.003	0.001 ± 0.001
2010	1.38 ± 0.32	2.48 ± 0.52	0.22 ± 0.03	0.03 ± 0.03	0.004 ± 0.003	0.001 ± 0.001

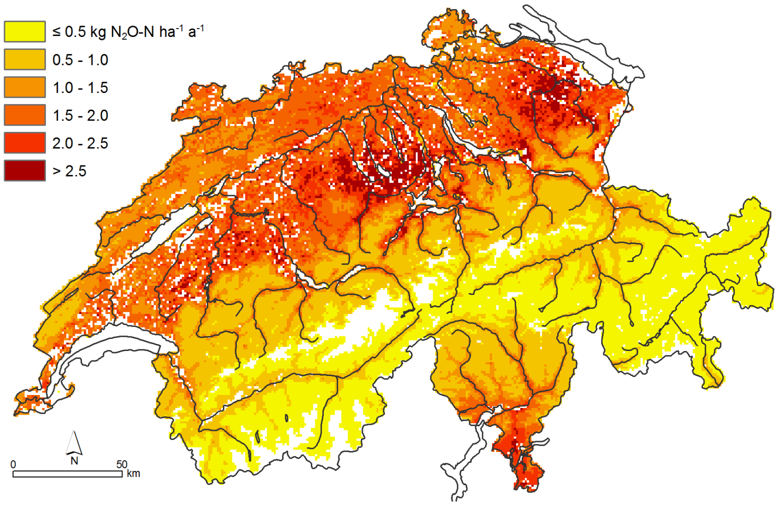

Figure 2.3: Indirect N_2O emissions of (semi-)natural ecosystems in Switzerland aggregated per km^2 in the year 2010. White pixels represent areas with ecosystem types not selected in the present study (e.g., urban areas, cropland, rocks).

3.2. NO emission

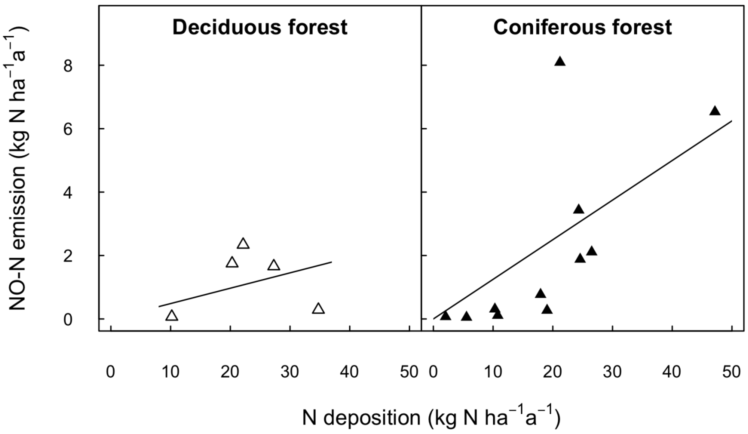

Figure 2.4: Indirect NO emissions in dependence of total N deposition into deciduous and coniferous forests. NO emissions and N deposition expressed in kg N ha^{-1} a^{-1}. The linear regression was significant in coniferous forests (n = 11, R^2_{adj} = 0.64, $p < 0.01$) and marginally significant in deciduous forests (n = 5, R^2_{adj} = 0.51, p = 0.066).

NO emission was related significantly and positively to atmospheric N deposition in coniferous forests, and marginally significant in deciduous forests (Fig. 2.4). Data of deciduous forests were included to roughly estimate their indirect NO emissions. As stated for the N_2O emissions, annual mean air temperature or annual precipitation did not explain indirect NO emission rates in the different ecosystems. The emission factor was 2.6-times higher in coniferous than in deciduous forests (Table 2.1). The approach by Skiba et al. (1997) resulted in very low NO-N emissions: in the year 2010, only 0.03 Gg NO-N were emitted in grassland, and 0.001 Gg NO-N were emitted in wetlands in Switzerland. Hence, 99% of all indirect NO-N emissions of (semi-)natural ecosystems were calculated to be emitted from forests, around 1% from grassland, and less than 0.1% from wetlands. In the year 1990, Swiss (semi-)natural ecosystems emitted 3.09 ± 0.65 Gg NO-N, in 2000 2.61 ± 0.55 Gg NO-N, in 2007 2.53 ± 0.53 Gg NO-N and in 2010 2.51 ± 0.53 Gg NO-N (Table 2.2). In line with the indirect N_2O emissions, indirect NO emissions were highest in areas with high atmospheric N deposition and much lower in remote, mountainous areas (Fig. 2.5).

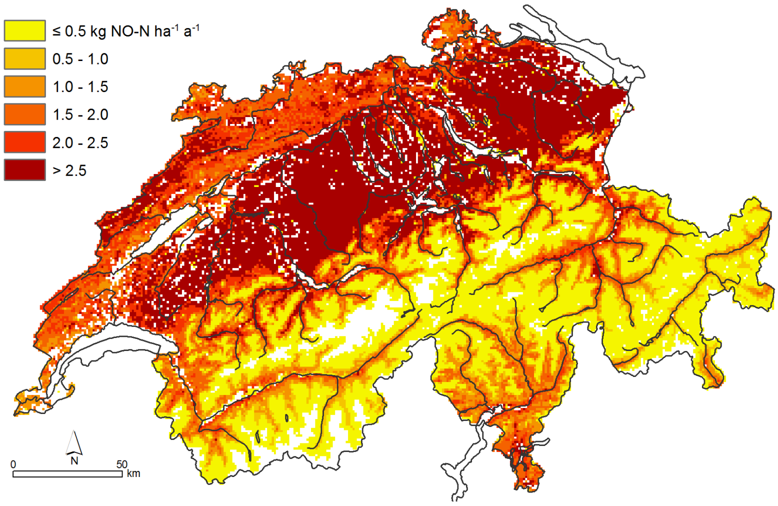

Figure 2.5: Indirect NO emissions of (semi-)natural ecosystems in Switzerland aggregated per km^2 in the year 2010. NO emissions of grassland and wetlands were calculated following Skiba et al. (1997). White pixels represent ecosystem types not considered here.

4. Discussion

We have shown that owing to increased atmospheric N deposition (semi-)natural ecosystems are a relevant source of indirect N_2O and NO emissions in Switzerland. Their source strength corresponded to 21% of the total national N_2O emissions and 10% of the total national NO_x emissions in the year 2010 (total N_2O and NO_x emissions: FOEN, 2012a; FOEN, 2012b). However, over the past 20 years N deposition into (semi-)natural ecosystems decreased by 20% leading to a reduction in the same range of indirect NO and N_2O emissions.

In the three (semi-)natural ecosystems studied here the correlations between atmospheric N deposition and indirect N_2O or NO emissions were significant and positive or marginally significant (deciduous forests), except for wetlands. These positive correlations have been confirmed by several authors (Butterbach-Bahl et al., 1998; Gasche and Papen, 2002; Lohila et al., 2010; Papen and Butterbach-Bahl, 1999; Pilegaard et al., 2006; Skiba et al., 2012). We found that coniferous forests emit more NO than N_2O induced by N deposition, whereas in deciduous forests it is vice versa (Denier van der Gon and Bleeker, 2005; Gasche and Papen, 2002). Gasche and Papen (1999) hypothesized that these differences in NO emissions may also result from higher NO consumption rates in deciduous forests. Pilegaard et al. (2006) explained the higher NO emission of coniferous compared to deciduous forests by the lower soil moisture and the well-aerated, thick litter layer in coniferous forests, which may favour nitrification and thus, NO production. In deciduous forests the litter layer is often more compact and moist, thus denitrification takes over, triggering N_2O production.

Depending on the ecosystem, the N_2O emission factors found in the present study are 2–8 times higher compared to the fertilizer-induced N_2O emission factor (0.91%) found by Stehfest and Bouwman (2006) for agricultural land. Nonetheless, according to the '2006 IPCC Guidelines for National Greenhouse Gas Inventories' it is suggested to calculate the indirect N_2O emissions of soils by means of the fertilizer-induced emission factor for direct N_2O emissions of agricultural soils. In case EF_4 is used to estimate indirect N_2O emissions for Swiss forests (N deposition x EF_4), the resulting emissions are 4.4 times lower compared to our estimate for the year 2000. The process-oriented 'PnET-N-DNDC' model by Kesik et al. (2005) resulted in a 2.4 times higher N_2O emission for Swiss forests for the year 2000 compared to the calculation using EF_4, but is 1.8 times lower compared to our approach. The latter difference may be explained by the fact that our input data (N deposition, land use statistics) are in a 1-ha-grid, whereas Kesik et al. (2005) used the 50 x 50 km grid by EMEP for soil properties and wet N deposition. The low spatial resolution led to satisfying results for forests in the lowlands, where N deposition, soil properties and topography were similar over large areas. However, in topographically complex areas such as in Switzerland, the model is considered too coarse to precisely predict N emissions of forests. Even though the total wet N deposition for Switzerland modelled by EMEP was in good agreement, the N input into forests was clearly undervalued by the EMEP data. Besides, the difference in the spatial resolution, the model of Kesik et al. (2005) is process-oriented and includes more parameters (i.e., meteorological data, soil properties) adding to differences in the model output. However, the results of both studies illustrate, in line with Papen and Butterbach-Bahl (1999), that indirect N_2O emissions of forests are currently underestimated by the approach proposed in the IPCC guidelines.

Referring to NO emission, Gasche and Papen (1999) reported emission factors that were in the range of ours. Our model resulted in 17-fold higher NO emissions of (semi-)natural ecosystems compared to results employing to the 'simpler methodology' (based on data of agricultural soils; EMEP/EEA, 2013). In case this approach was applied to Swiss forests, the NO emission would be 22 times lower than in our approach and 5 times lower compared to the results of Kesik et al. (2005) for the year 2000. Again, the difference between the latter and our results may be explained by differences in spatial resolution and the model type. We deduce that the indirect NO emissions of (semi-)natural ecosystems are largely underrated by the 'simpler methodology', the uncertainty of these emissions is still considerable, and only few long-term data have been available so far.

The approaches of the IPCC guidelines and the EMEP/EEA guidebook underestimate the indirect N_2O and NO emissions of (semi-)natural ecosystems, since they are based on data for agricultural systems and do not account for differences between ecosystems. In agricultural systems, fertilization is adjusted to the nutritional demand of crops in order to optimize yield and productivity. Overall, N fertilizer addition rates are much higher in agriculture compared to atmospheric N deposition in (semi-)natural ecosystems. For instance, in Switzerland oil seed rape and wheat are on average fertilized with 140 kg N ha^{-1} a^{-1}, and intensive grassland with up to 165 kg N ha^{-1} a^{-1} depending on the number of cuts (Flisch et al., 2009), whereas the atmospheric N deposition (for the year 2010) was on average 23.6 kg N ha^{-1} a^{-1} in forests and 8.5 kg N ha^{-1} a^{-1} in extensively used grassland considered here. The net N withdrawal by tree harvest is assumed to be 9 kg N ha^{-1} a^{-1} in *Fagus* dominated forests and 6.4 kg N ha^{-1} a^{-1} in *Picea* dominated forests (calculated according to UNECE, 2004) and hence, much lower than in agricultural systems (c. 100 kg N ha^{-1} a^{-1} in a Danish conventional arable farming system; Jarvis et al., 2011). The fraction of N retained by European forests at a N deposition range of 15–40 kg N ha^{-1} a^{-1} is supposed to be 40–100% and in areas with N deposition >40 kg N ha^{-1} a^{-1} N-retention is even lower (0–70%; de Vries et al., 2006). Indeed, de Vries et al. (2003) have reviewed N leaching to increase due to nitrogen deposition and to frequently exceed 4 kg N ha^{-1} a^{-1} at medium N deposition of 20 kg N ha^{-1} a^{-1} in European forests. This demonstrates that, in contrast to agricultural systems, these forests have already been saturated at a low N input. Referring to N fluxes, agricultural systems and (semi-)natural ecosystems are very different, thus the approaches of IPCC and EMEP/EEA need to be adapted for (semi-)natural ecosystems. Furthermore, the indirect N_2O and NO emissions modelled in the present study were similar to the N losses through denitrification used for CLN modelling (in the range of 0.1–3 kg N ha^{-1} a^{-1}; UNECE, 2004).

5. Conclusions

The effect of atmospheric N deposition on indirect N_2O and NO emissions differs among (semi-)natural ecosystems and is up to 8-fold higher for N_2O emissions and up to 28-fold higher for NO emissions compared to agricultural systems. Thus, we advocate not to use models based on agricultural data and N emissions per country, especially not for countries with a high proportion of forests, extensively used, perennial grassland, and wetlands such as Switzerland. Here, a site- and ecosystem-specific model based on atmospheric N deposition, improves the accuracy of the national emission inventories.

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Supplementary material

Table S2.1: Field data of N_2O-N, NO-N emissions and N deposition data (inc. modelled data, expressed in kg N ha^{-1} a^{-1}) from the literature.

Vegetation (Country)	N-deposition	N_2O-N	NO-N	Air temp. (°C)	Precipitation (mm)	Author (year)
Coniferous forest						
(DK)	14.56	0.77	n.a.	8.6	756	Ambus and Christensen (1995)
(US)	6.00	0.48	n.a.	9.7	890	Ambus and Robertson (2006)
(US)	2.20	0.02	n.a.	7.4	1120	Bowden et al. (1991)
(IE)	10.45^{2}	0.07	n.a.	9.5	800	Butterbach-Bahl et al. (1998)
(DE)	19.04^{3}	0.41	0.27	7.3	545	Butterbach-Bahl et al. (2002)
(DE)	17.95^{3}	0.85	0.77	7.3	550	Butterbach-Bahl et al. (2002)
(DE)	24.58^{3}	2.63	1.88	7.3	561	Butterbach-Bahl et al. (2002)
(DE)	26.51^{3}	2.80	2.11	7.3	500	Butterbach-Bahl et al. (2002)
(DE)	24.34^{3}	1.37	3.43	7.3	500	Butterbach-Bahl et al. (2002)
(SE)	15.66^{3}	0.06	n.a.	6.4	1100	Gundersen et al. (1998)
(DK)	27.71^{3}	0.04	n.a.	9.0	860	Gundersen et al. (1998)
(GB)	16.87^{3}	0.50	n.a.	8.8	1850	Gundersen et al. (1998)
(SE)	10.78^{2}	n.a.	0.11	n.a.	n.a.	Johansson (1984)
(SE)	2.05^{2}	n.a.	0.07	3.8	600	Johansson (1984)
(AT)	5.55	0.76	0.05	7.0	1672	Kesik et al. (2005)
(DE)	21.20	1.00	8.09	8.6	849	Kesik et al. (2005)
(FI)	0.10	0.05	n.a.	4.2	589	Kesik et al. (2005)
(HU)	10.30	1.75	0.31	8.6	744	Kesik et al. (2005)
(IT)	4.60	0.20	n.a.	14.6	922	Kesik et al. (2005)
(NE)	47.15	0.22	6.53	10.4	769	Kesik et al. (2005)
(SE)	14.46	0.07	n.a.	n.a.	n.a.	Klemedtsson et al. (1997)
(SE)	14.46	0.12	n.a.	n.a.	n.a.	Klemedtsson et al. (1997)
(GB)	9.33^{2}	0.35	n.a.	n.a.	n.a.	Skiba et al. (1996)
(GB)	9.33^{2}	0.13	n.a.	n.a.	n.a.	Skiba et al. (1996)
(GB)	9.33^{2}	0.31	n.a.	n.a.	n.a.	Skiba et al. (1996)
(GB)	8.72^{2}	0.36	n.a.	n.a.	n.a.	Skiba et al. (1996)
Deciduous forest						
(DK)	14.56^{2}	0.80	n.a.	8.6	756	Ambus and Christensen (1995)
(US)	6.00	0.31	n.a.	9.7	890	Ambus and Robertson (2006)
(SE)	8.66^{2}	2.00	n.a.	5.6	662	von Arnold et al. (2005)
(US)	2.20	0.04	n.a.	7.4	1120	Bowden et al. (1991)
(HU)	8.50^{2}	0.30	n.a.	9.6	690	Czóbel et al. (2010)
(HU)	8.64^{2}	2.42	n.a.	5.7	780	Czóbel et al. (2010)
(IT)	8.35	0.35	n.a.	14.4	834	Kesik et al. (2005)
(IT)	8.35	0.22	n.a.	14.4	834	Kesik et al. (2005)
(DE)	22.15	3.32	2.34	8.1	886	Kesik et al. (2005)
(DE)	20.30	1.13	1.75	9.0	812	Kesik et al. (2005)
(AT)	10.20	1.39	0.07	8.7	818	Kesik et al. (2005)
(HU)	7.60	1.93	n.a.	8.6	744	Kesik et al. (2005)
(AT)	27.28	4.61	1.66	9.9	779	Kesik et al. (2005)
(DK)	34.75	0.71	0.29	8.7	773	Kesik et al. (2005)
(DE)	23.80	0.40	n.a.	8.1	679	Mogge et al. (1998)
(GB)	9.33^{2}	0.60	n.a.	n.a.	n.a.	Skiba et al. (1996)
(GB)	5.71^{2}	0.66	n.a.	n.a.	n.a.	Skiba et al. (1996)
Grassland						
(CH)	10.00^{1}	0.33	n.a.	4.2	1336	Bühlmann et al., unpubl.
(CH)	8.90^{1}	0.35	n.a.	4.2	1336	Bühlmann et al., unpubl.
(FR)	10.49^{2}	0.72	n.a.	8.7	780	Cantarel et al. (2011)
(HU)	9.68^{2}	0.45	n.a.	10.5	587	Czóbel et al. (2010)
(HU)	10.67^{2}	0.38	n.a.	10.4	562	Czóbel et al. (2010)
(CH)	5.60^{1}	0.39	n.a.	n.a.	970	Hartmann et al. (2013)
(GB)	9.33^{2}	0.88	n.a.	n.a.	n.a.	Skiba et al. (1996)
Wetland						
(EE)	8.00	0.42	n.a.	4.3	889	Carter et al. (2012)
(SE)	15.00	-0.02	n.a.	7.1	626	Carter et al. (2012)
(GB)	6.57^{2}	-0.14	n.a.	8.5	1092	Carter et al. (2012)
(HU)	8.50^{2}	0.81	n.a.	9.6	690	Czóbel et al. (2010)
(FI)	1.38	0.23	n.a.	n.a.	n.a.	Lohila et al. (2010)
(FI)	4.88^{2}	0.04	n.a.	n.a.	n.a.	Martikainen et al. (1993)
(FI)	4.88^{2}	0.04	n.a.	n.a.	n.a.	Martikainen et al. (1993)
(FI)	4.88^{2}	0.04	n.a.	n.a.	n.a.	Martikainen et al. (1993)
(FI)	4.88^{2}	0.04	n.a.	n.a.	n.a.	Martikainen et al. (1993)

^{1} Modelled in this study; ^{2} model data by EMEP (EMEP, 2011); ^{3} N in throughfall converted to total N deposition; n. a.: no data available

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Chapter 3

Alnus viridis expansion contributes to excess reactive nitrogen release, reduces biodiversity and constrains forest succession in the Alps

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Abstract

Reduction in land use and complete land abandonment are widespread in mountainous regions and are mainly driven by socio-economic factors. Following land-use decline, shrubs and trees expand rapidly into montane and subalpine grassland and alter ecosystem properties at a large scale. In particular, the N_2-fixing shrub *Alnus viridis* is currently spreading at a breath-taking speed and thereby reduces biodiversity, leads to substantial reactive nitrogen enrichment and suppresses species succession towards coniferous forests across large areas in the Alps. In addition, this shrub vegetation neither protects against avalanches nor does it secure slopes from erosion. The expanding, monotonous *A. viridis* shrubland is impenetrable for hikers and diminishes scenic beauty and touristic value of the landscape. Actions and management adaptations are needed to halt the expansion of *A. viridis.* Goats and the traditional sheep breed Engadine sheep proved to be very effective in preventing and reverting shrub expansion because of their specific browsing behaviour.

1. Introduction

Around 5,000 years ago, settlers started to clear forests in the Alps, which led to a mosaic of pastures and meadows (Bätzing, 2005). Over millennia, this rangeland has been grazed by domestic and wild animals, used for haymaking and hence, served for fodder and food production. Human activity has been shaping the landscape, and the type of land use has been affecting the distribution and composition of plant communities (Lueth et al., 2011; Tasser and Tappeiner, 2002). Modernisation of agriculture has mostly been driven by socio-economic factors and started in mountainous regions during the mid-twentieth century (Rey Benayas et al., 2007). It has fundamentally changed agriculture: on one hand, there has been – and still is – intensification on productive and easily accessible agricultural land (Fischer et al., 2008; Tasser and Tappeiner, 2002). On the other hand, poorly accessible or low-productive land has been less intensively used or has been completely abandoned, i.e. meadows have been converted to pastures and former pastures have often been given up. Overall, land abandonment is the dominant process of land-use change in the Alps and between 1985 and 2009, 42.5% of montane farms disappeared in Switzerland (Federal Statistical Office of Switzerland, 2013). The area of montane and alpine pastures decreased drastically, whereas the forest area increased by at least 40% in Switzerland during the past 150 years (percentage values are here calculated without shrubland; Brändli, 2010).

This massive land-use change has substantial implications for ecosystem functioning. Ecosystem properties that have developed over centuries of human influence are being altered within a few decades only, with the expansion of forests and shrubland exerting the most significant change. Beside shrubland and forests, dwarf shrubs of the genus *Calluna*, *Vaccinium* and *Rhododendron* are rapidly expanding into montane and alpine grassland and often form dense carpets (Bischof, 1984; Tasser and Tappeiner, 2002).

From 1993/95 to 2004/06, shrubs have overgrown an area of around 11,700 ha in the Swiss Alps (Brändli, 2010). In Switzerland, *Alnus viridis* [Chaix] DC (green alder) contributes 71% to the shrubland and has overgrown an area of 46,000 ha by 2004/06. Similar expansions of *A. viridis* were observed in the French Alps, for example in the Vanoise National Park, where 7% of the montane and subalpine area was covered by *A. viridis* shrubland in the late 90s (Richard, 1989). Not only the Alps are affected by expanding *Alnus* shrubs: *Alnus viridis* ssp. *fruticosa* has been reported to expand in Alaska and Canada (Myers-Smith et al., 2011) and in Siberia (Frost and Epstein, 2014).

Alnus viridis is an early successional shrub that occurs naturally in disturbed habitats such as avalanche tracks, moist ravines or edges of small, steep creeks

(Bischof, 1984). This species belongs to the native flora of the Alps (David, 2010) and has a wide elevational range from about 900 m a.s.l. to the treeline (2,000 m a.s.l; Richard, 1967). *Alnus viridis* lives in symbiosis with the actinomycete *Frankia alni*, forming typical root nodules within these, protected from oxygen, N_2 is transformed into ammonia by means of the nitrogenase enzyme complex (Dawson, 2008). For *Alnus viridis* ssp. *sinuata* and *Alnus viridis* ssp. *crispa*, nitrogen fixation rates of 20 up to 62 kg N ha^{-1} a^{-1} have been reported (Binkley, 1981). These subspecies are native to North America, have similar stature and habitat preferences as *Alnus viridis* [Chaix] DC. Ongoing research in the Swiss Alps revealed that the N input of *A. viridis* is of a similar magnitude as of the N-American relatives (Bühlmann et al., unpubl.; Hiltbrunner et al., 2014) and thus, by far exceeds the critical load of nitrogen for these former mountain grasslands (Bobbink et al., 2011). The nitrogen fixation rates are considerably larger than the current total input by atmospheric nitrogen deposition, which is in the range of 5–10 kg N ha^{-1} a^{-1} for the alpine and 10–15 kg N ha^{-1} a^{-1} for the montane belt in Switzerland (Hiltbrunner et al., 2005; modelled data provided by Meteotest, Switzerland). Owing to its ability to symbiotically fix nitrogen, *A. viridis* grows more rapidly than most other woody species: its rate of spreading is two to three times faster than the current forest expansion in Switzerland (calculated from data of Brändli, 2010). The sudden release of open terrain by reducing centuries-old land care in the immediate vicinity of natural *A. viridis* habitats contributes to the extraordinary spreading of this species across the Alps.

2. Changes in ecosystem properties

2.1. Biodiversity

Montane and alpine grassland ecosystems are hotspots for plant species diversity (Fischer et al., 2008) and are richer in plant and animal species compared to montane shrubland or forests (Koch et al., 2013; Zoller et al., 1984). Either intensification or abandonment causes the montane land cover to become more uniform and, thus, reduces plant species richness at different spatial scales (Fischer et al., 2008; Niedrist et al., 2009; Spehn et al., 2006). This loss in biodiversity is amplified by the excessive nitrogen input via the *Alnus–Frankia* symbiosis: increased nitrogen availability leads to a decline in plant species richness because nitrophilic, fast-growing species outcompete slow-growing species (Suding et al., 2005). As *A. viridis* shrubs expand, only a few plant species can cope with the shady, cool and moist conditions beneath the *Alnus* canopy, forming a rather species-poor, dense understory vegetation with species such as *Adenostyles alliariae, Cicerbita alpina, Rumex alpestris, Achillea macrophylla* and *Thalictrum aquilegiifolium.* The intermediate mosaic stage of grassland mixed with a few *A. viridis* shrubs is often temporarily favourable for plant species richness, but as soon as the *A. viridis* cover exceeds 50% of the total cover, plant species richness drops to less than half of the former plant diversity in grassland (Anthelme et al., 2001; Bischof, 1984). As a consequence of the loss in plant species diversity, the arthropod diversity declines as well (including butterflies; Anthelme et al., 2001; Zoller et al., 1984). In addition, dense *A. viridis* shrubland is not suitable for nesting for the endangered species *Tetrao tetrix* (black goose) and does not provide enough arthropods for their chicks during summer.

2.2. Soil, water and climate

Nitrate leaching to streamlets and groundwater generally occurs as soon as soils become nitrogen saturated (Aber et al., 1989). Soils are saturated when their storage capacity for nitrogen is exceeded and the availability of nitrogen compounds exceeds the total combined plant and microbial nutritional demand. For *A. rubra*, which is, in contrast to *A. viridis*, a tall tree, nitrogen leaching rates (in form of nitrate) of up to 50 kg N ha^{-1} a^{-1} were reported (Binkley et al., 1992; Compton et al., 2003). Measurements with suction cups in soils under *Alnus viridis* in central Switzerland revealed very high concentrations of nitrate in soil water below the main rooting horizon, frequently exceeding the Swiss threshold for freshwater (25 mg NO_3^- L^{-1}), whereas the soil solution in adjacent pastures was nearly free of nitrate (Bühlmann et al., unpubl.). This indicates that *A. viridis* stands are nitrogen saturated and nitrate leaching does occur at high rates, whereas leaching losses are commonly low in montane coniferous climax forests (zero to 2.2 kg N ha^{-1} a^{-1} in *Pinus sylvestris* and *Picea abies* forests in the Swiss Alps; Thimonier et al., 2009). Nitrogen enrichment leads to soil acidification through proton production during nitrification and NH_4^+ uptake by plants, and also to losses of base cations during leaching of nitrate. Soils under *A. viridis* shrubland have indeed been found to be more acidic than soils in adjacent forest and grassland (Bühlmann et al., unpubl.; Podrazsky and Ulbrichova, 2003). In addition to N losses through leaching, extremely high nitrous oxide emissions of more than 4 kg N_2O-N ha^{-1} season^{-1} have been measured in *A. viridis* shrubland at 1,500 m a.s.l. in central Switzerland, measured from June to end of September. Adjacent pastures emitted 35 times less of this potent greenhouse gas during the same period (Bühlmann et al., unpubl.).

2.3. Suppressed succession towards forest

Vegetation succession starts immediately after land abandonment and natural climax forests establish over time, in particular, below or close to the natural tree line (Tasser and Tappeiner, 2002; Wallentin et al., 2008). The presence of *A. viridis* stands prevents such succession towards montane forest. Tree seedlings, for instance of *Larix*, *Pinus* and *Picea*, are unable to establish under *A. viridis* shrubs and its vigorous herbaceous understory (Bischof, 1984). In the central Swiss Alps less than 5% of *A. viridis* shrubland developed into forest within 75 years (Huber and Frehner, 2012). Experiences of local farmers show that clear cutting does neither help turning this shrubland into a forest nor does it contribute to reverting the bush into grassland. After clear cutting, *A. viridis* shoots re-sprout vigorously from the rootstock. In addition, the dense understory is maintained over decades due to the high amount of nitrogen in the soil. Thus, without continuous and adapted management, forests cannot develop and replace *A. viridis* thickets once established. Moreover, the encroachment of former grassland by *A. viridis* is also problematic because this shrub does not fulfil the protective function of a montane forest against avalanches, erosion and shallow landslides (Bischof, 1984; Caviezel et al., 2014; Tasser et al., 2003).

3. Economic consequences

The rapid expansion of *A. viridis* does not only affect ecosystem properties at a large scale, but also exerts economic drawbacks. Open landscapes are considered as a valuable natural resource for Swiss tourism worth about 68–79 billion CHF and therefore, contribute substantially to the main source of income especially in mountainous regions depending on tourism (Econcept, 2002). Traditionally used agricultural land is diverse and is regarded more attractive by visitors and locals compared to monotonous forests or shrubland (Hunziker, 2001; Schirpke et al., 2012). The impenetrable thickets of *A. viridis* shrubland reduce scenic beauty and thus, the touristic value of the landscape. Depending on climate change and the economic situation, montane and subalpine agricultural land may become more important for forage and food production in the future (Schirpke et al., 2012). Farms with a high share of montane grassland will suffer less from summer drought, such as that in the year 2003, compared to farms situated exclusively in the lowlands (Finger et al., 2013). It is predicted that drought spells will occur more frequently in the future (Seneviratne et al., 2012) and thus, the importance of these high elevation rangelands for fodder provision will rise. It is very time consuming and labour-intensive to clear *A. viridis* shrubland and consequently, the loss of this valuable montane grassland is almost irreversible. Hence, future generations lose opportunities to adapt to and to mitigate future climatic and economic constraints.

4. Management options

Preservation of these montane cultural landscapes and stopping further encroachment by *A. viridis* is best achieved by sustainable land use. However, regular haymaking and thereby, suppression of shrub and tree colonisation is labour-intensive and more expensive compared to animal browsing, particularly on steep slopes (Dux et al., 2009). Cultural landscapes can be kept open by goats and special breeds of sheep, which are browsing on the bark of shrubs and trees. Removing the bark and partly injuring the xylem, cause woody plants to die back. Goats and the traditional sheep breeds Engadine sheep (in German: 'Engadinerschaf'; commonly raised in Switzerland) and the alpine stone sheep (in German: 'Alpines Steinschaf'; occurring in southern Germany and Austria; Jaritz, 2010) browse on woody plants. The most common sheep breeds (e.g. 'weisses Alpenschaf') do not feed on the bark of woody plants at all. Engadine sheep have been shown to be very efficient: after one single summer grazing period, 46% of *A. viridis* branches died and in recently encroached pastures even 76% branches died back (MSc project by T. Zehnder, cited in Bühlmann et al., 2013). Furthermore, meat of Engadine sheep is of excellent quality with a low fat content (Willems et al., 2013). Therefore, a 'value chain' could easily be generated by meat products of this sheep breed and as a side effect these sheep help to keep old cultural land open. We highly recommend increasing the numbers of goats and Engadine sheep either by means of subsidies or by establishing 'value chains' to reduce shrub encroachment and forest expansion, and as a consequence assist in the protection of biodiversity, soil and water quality and scenic beauty in the Alps.

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Die Verbuschung des Alpenraums durch die Grünerle

Durch den Rückzug der Landwirtschaft aus dem Berggebiet breiten sich Grünerlen rasant aus. Diese Büsche überwachsen artenreiche Wiesen und Weiden, verhindern die Rückkehr des Bergwaldes und führen zu Stickstoffbelastungen für Gewässer und Boden. Die Ausbreitung der Grünerle kann besonders effektiv durch Ziegen und Engadiner Schafe gebremst werden.

Verbuschung und Verwaldung des Alpenraums

Vor rund 5000 Jahren begann die Besiedelung des damals weitgehend bewaldeten Alpenraumes. Durch grossflächiges Roden wurden Wiesen und Weideland geschaffen, welche für die Menschen in den Bergregionen über Jahrhunderte die Ernährungsgrundlage darstellten und bis heute das Landschaftsbild prägen. Im Zuge der Modernisierung seit dem 20. Jahrhundert suchen die Menschen den Aufwand zu verringern: Gut erreichbare Flächen werden intensiver genutzt, abgelegene Flächen werden extensiviert. Schnittwiesen werden in Weiden umgewandelt und frühere Weiden ganz aufgegeben. Das führt im waldfähigen Gebiet zur Wiederbewaldung oder Verbuschung.

Gehölze im Vormarsch

Wald und Gebüschwald nehmen in der Schweiz seit 150 Jahren zu; heute so schnell wie nie zuvor. Besonders betroffen ist der Alpenraum, wo am meisten landwirtschaftliches Land aufgegeben wird. Nicht genutztes alpwirtschaftliches Land kann bereits nach 20 Jahren komplett von Sträuchern oder Bäumen überwachsen sein. Beunruhigend schnell ist vor allem die Ausbreitung des Gebüschwaldes: Im Alpenraum werden gemäss dem dritten Schweizerischen Landesforstinventar jährlich 1000 ha von Gebüschwald überwachsen. Dieser Gebüschwald besteht in der Schweiz zu über 70 % aus Grünerlen, und im Alpenraum kann der Anteil 85 % erreichen. Es sind aber nicht nur Wald und Gebüschwald, die sich auf Kosten jahrhundertealter Kulturlandschaft ausbreiten, sondern auch andere Pflanzen wie der Adlerfarn, die Alpenrose oder die Besenheide.

Konsequenzen für Biodiversität und Umwelt

Die Grünerle, auch Alpenerle genannt, ist eine einheimische Pflanze und wächst üblicherweise in Lawinenzügen und Bachrunsen oberhalb 1100 m ü. M. Vielerorts kommt sie bis zur alpinen Wald- und Baumgrenze vor. Im Alpenraum ist die Grünerle seit jeher vorhanden, blieb aber auf ihre angestammten Nischen beschränkt. Erst durch den schnellen Rückzug des Menschen aus den Berghängen begann sie sich schlagartig auszubreiten. In der Schweiz ist die Ausbreitung momentan rund drei- bis viermal schneller als beim Wald und kommt dadurch einer natürlichen Wiederbewaldung zuvor. Die massive Zunahme der Grünerlen geschieht im ganzen Alpenbogen. Ähnliche Phänomene sind auch aus Alaska und dem Himalaya bekannt.

Reduzierte Biodiversität

Die Grünerle lebt in Symbiose mit Stickstoff-fixierenden Bakterien (Bildung von Wurzelknöllchen, Abb. 1). Dies ist ein Grund für ihre schnelle Ausbreitung. Diese Bakterien wandeln Luftstickstoff so um, dass er von den Pflanzen als Nährstoff, quasi als Dünger, genutzt werden kann. Durch die Stickstofffixierung wird zudem mehr Stickstoff verfügbar als die Grünerle dauerhaft aufnehmen kann. Somit werden auch andere Pflanzen im Unterwuchs gedüngt.

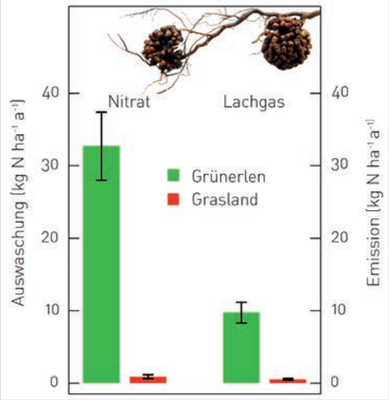

Abb. 1: Nitratauswaschung und Lachgasemission sind in Grünerlenbeständen massiv höher als im Grasland (Mittelwert ± SE). Die Stickstofffixierung findet in den Wurzelknöllchen statt.

Jedes Ökosystem wird durch erhöhte Stickstoffmengen stark verändert. Einzelne besonders wüchsige Pflanzen werden stark gefördert und verdrängen viele andere Arten. So kommen im Unterwuchs von Grünerlen meist nur noch relativ wenige Pflanzenarten wie der graue Alpendost oder der Alpenmilchlattich vor (Abb. 2). Bedecken Grünerlen mehr als die Hälfte einer Fläche, reduziert sich die Pflanzendiversität um rund die Hälfte, und Käfer, Heuschrecken, tagaktive Schmetterlinge und andere Insekten werden deutlich seltener. Birkhühner finden weniger Nahrung in Grünerlen als auf Wiesen und nisten nicht im dichten Grünerlengebüsch.

Kleinflächige Grünerlengebüsche können durchaus zur Vielfalt einer Landschaft beitragen. Herrscht aber die Grünerle quasi als Monokultur über grosse Flächen vor, reduziert sich auch die Lebensraumvielfalt. Da artenreiche Wiesen und Weiden im Alpenraum deutlich häufiger sind als im Flachland, ist der Verlust an Biodiversität erheblich.

Boden, Gewässer und Klima werden belastet

Der Überschuss an Stickstoff wird über mehrere Wege aus den Erlenbeständen freigesetzt, zwei sind besonders problematisch: Erstens wird er in Form von Nitrat ausgewaschen, zweitens gelangt er als Lachgas in die Luft (Abb. 1).

Zwischen 15 bis 30 kg Nitrat-Stickstoff pro Hektare und Jahr werden so ausgewaschen. Dabei gehen auch weitere wichtige Nährstoffe wie basische Kationen verloren. Im Vergleich zum Grünerlengebüsch wird aus einer Wiese oder einem Bergwald kaum Nitrat ausgetragen. Infolge der Stickstoffanreicherung haben viele Prozesse im Boden (Nitrifikation, Ammoniumaufnahme durch Pflanzen) eine versauernde Wirkung. Deshalb versauert der Boden im Grünerlengebüsch zunehmend.

Erste Lachgasmessungen in Grünerlenbeständen zeigen 35-mal höhere Emissionsraten als in Wiesen. Lachgas ist durch seine atmosphärische Verweilzeit von durchschnittlich 114 Jahren ein fast 300mal stärkeres Treibhausgas als CO_2. Die jährlichen Emissionen einer Hektare Grünerlengebüsch sind für das Klima etwa gleich schädlich wie der CO_2-Ausstoss von 15 000 gefahrenen Autokilometern.

Kein Wald

Erwünscht wäre, dass auf ehemaligen Mähwiesen und Weiden, falls sie nicht erhalten werden können, wieder der Wald einzieht. Auch aus einem Gebüschwald kann im Laufe der Zeit ein Wald entstehen. Nicht jedoch bei Grünerlen: Sämlinge von Nadelbäumen können im Grünerlenbestand und in deren dichtem Unterwuchs nicht aufkommen. Selbst das Abschneiden der Grünerlen nützt wenig, denn sie treiben immer wieder aus dem Stock aus, und das hohe Stickstoffangebot im Boden bringt wiederholt eine üppige Krautschicht hervor. Ohne jahrzehntelange Pflege kann unter diesen Bedingungen kein Wald aufkommen. Zudem bietet die Grünerle nicht den Erosions- und Lawinenschutz des Bergwaldes. Bei Vernässung fördert sie sogar das blockweise Abrutschen des wertvollen Oberbodens.

Ökonomische Konsequenzen

Beeinträchtigtes Landschaftsbild

Das Landschaftsbild der Schweiz ist eine natürliche Ressource von beachtlichem Kapitalwert. Allein für den Schweizer Tourismus, dem grössten Arbeitgeber im Alpenraum, wird der Wert des Landschaftsbildes auf über 68 Milliarden Franken geschätzt. Die Ausbreitung der Grünerle verändert das Landschaftsbild stark. Im Vergleich zu offenen, kleinräumig strukturierten Berglandschaften erachten Touristen und Einheimische grossflächige, monotone und undurchdringliche Grünerlengebüsche als weniger schön und wertvoll. Die Verbuschung führt somit zu einem Verlust des Landschaftswertes. Alpwirtschaftsland kann im Zusammenhang mit dem Klimawandel als Ausgleichsfläche für trockenes Land im Tal wieder an Wert gewinnen. Einmal verbuscht, sind solche Flächen nur schwer zurückzugewinnen und schränken somit den Handlungsspielraum späterer Generationen ein. Zudem verdunsten Grünerlengebüsche mehr Wasser als gepflegtes Weidland, was den Abfluss verringert und damit – wenn auch geringfügig – die mögliche Stromerzeugung im Sommer vermindert (Resultate des 2012 abgeschlossenen Nationalfondsprojekts VALUrsern).

Handlungsmöglichkeiten

Gesetzliche Rahmenbedingungen

Artikel 104 der Bundesverfassung verpflichtet die Schweiz zur «Erhaltung der natürlichen Lebensgrundlagen und zur

Abb. 2: Der dichte Grünerlen-Unterwuchs lässt kein Licht auf den Boden kommen.

ENGADINER SCHAFE

Das Engadiner Schaf bewährt sich nicht nur gegen die Verbuschung im Berggebiet, sondern hat auch aus ökonomischer Sicht interessante Eigenschaften. Die Schafrasse ist besonders robust und die Fleischqualität ist hoch. Unter den Schweizer Schafrassen ist sie Meisterin, was die Anzahl Lämmer pro Jahr betrifft. All dies dürfte das etwas geringere Schlachtgewicht mehr als kompensieren.

Pflege der Kulturlandschaft». Die Agrarpolitik 2014–2017 will dem vermehrt Rechnung tragen und setzt Anreize, welche die Kulturlandschaft des Sömmerungsgebietes offenhalten soll. Eine Studie von Agroscope hat aber ergeben, dass die vorgesehenen Massnahmen nicht ausreichen, um die Verwaldung und Verbuschung des Weidelandes zu stoppen. Um die Biodiversität der Alpen zu erhalten, sind schweizweit Massnahmen zu ergreifen und besonders artenreiche Flächen speziell zu schützen. Ebenfalls ist es der einheimischen Bevölkerung ein grosses Anliegen, dass die Verbuschung des hochgelegenen Kulturlandes gebremst wird.

Massnahmen zur Offenhaltung

Kulturland lässt sich nur durch Nutzung offenhalten. Mechanische Massnahmen (Rückschnitt, Mulchen) sind aufwändig und im steilen Gelände oftmals kaum möglich; sie lösen das Problem nicht dauerhaft. Ziegen und Engadiner Schafe haben hingegen die Eigenschaft, Triebe und Rinde von Sträuchern wie der Grünerle zu fressen, was zu deren Absterben ohne Stockausschlag führt (Abb. 3). Schon nach einer einzigen Weidesaison sind die Erfolge sichtbar. Vor allem Flächen, bei welchen das Einwachsen erst beginnt, können mit einer gezielten Beweidung offen gehalten werden. Andere Schafrassen hingegen fressen nur Gras und keine Rinde von Gehölzen. Ziegen und Engadiner Schafe sollten im Sömmerungsgebiet speziell gefördert werden, da sie sehr gute Dienste leisten im Einsatz gegen die Verbuschung von Grasland und für die Erhaltung der Biodiversität.

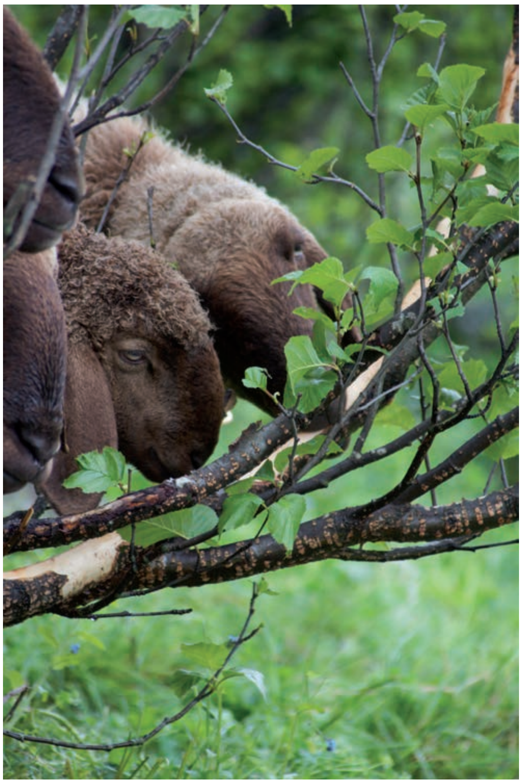

Abb. 3: Engadiner Schafe gehen den Grünerlen an den Kragen bzw. an die Rinde.

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Embroussaillement de l'arc alpin par l'aulne vert

En montagne, le retrait de l'agriculture a entraîné une prolifération rapide de l'aulne vert. Ce buisson recouvre des prairies et des pâturages riches en espèces, empêche le retour de la forêt de montagne et provoque une pollution des eaux et du sol par l'azote. La prolifération de l'aulne vert peut être efficacement enrayée par les chèvres et les moutons d'Engadine.

Embroussaillement et reboisement de l'arc alpin

Il y a quelque 5000 ans débuta la colonisation de l'arc alpin, jadis en grande partie couvert de forêts. Un défrichage à grande échelle permit de créer des prairies et des pâturages, qui fournirent pendant des siècles la base alimentaire des habitants de la montagne et ont caractérisé jusqu'à aujourd'hui le paysage rural. Depuis le XX^{e} siècle, la modernisation a entraîné une réduction du volume de travail : les surfaces faciles d'accès font l'objet d'une exploitation plus intensive, tandis que l'utilisation des parcelles isolées est extensifiée. Les prairies de fauche sont transformées en pâturages et les anciens pâturages sont totalement abandonnés. Il en résulte un reboisement ou un embroussaillement des surfaces potentiellement forestières.

Progression des buissons

La forêt et la forêt buissonnante s'étendent en Suisse depuis 150 ans, et aujourd'hui plus rapidement que jamais. Ce phénomène affecte en particulier l'arc alpin, où la déprise agricole est la plus marquée. Les terres non exploitées sous forme d'alpage peuvent être déjà complètement envahies par les arbres ou les buissons au bout de vingt ans. La vitesse de prolifération de la forêt buissonnante s'avère préoccupante : dans les régions alpines, 1000 ha sont recouverts par la forêt buissonnante chaque année, selon le troisième inventaire forestier national. Cette forêt se compose en Suisse d'aulnes verts à plus de 70 %, cette part pouvant atteindre 85 % dans l'arc alpin. Ce ne sont toutefois pas seulement la forêt et la forêt buissonnante qui se propagent au détriment d'un paysage rural séculaire, mais aussi des plantes telles que la fougère aigle, le rhododendron et la callune.

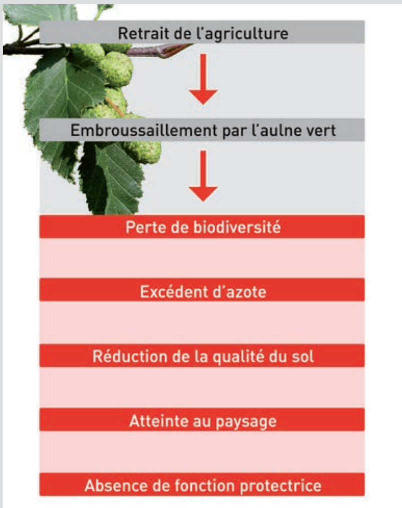
Conséquences pour la biodiversité et l'environnement

L'aulne vert, également appelé aulne des Alpes, est une plante endémique qui pousse en général dans les couloirs d'avalanche et les lits de ruisseaux asséchés, au-delà de 1100 m d'altitude. Souvent, on l'observe jusqu'à la limite alpine des forêts et des arbres. Dans l'arc alpin, l'aulne vert a toujours été présent, mais il se cantonnait à ses niches d'origine. Il a fallu que l'homme se retire rapidement des versants de la montagne pour qu'il commence à se répandre brusquement. A l'heure actuelle, en Suisse, sa propagation est trois à quatre fois plus rapide que celle de la forêt et anticipe ainsi un reboisement naturel. La prolifération de l'aulne vert affecte l'ensemble de l'arc alpin. Des phénomènes analogues se sont produits en Alaska et dans l'Himalaya.

Réduction de la biodiversité

L'aulne vert vit en symbiose avec des bactéries fixatrices d'azote (formation de nodosités racinaires, fig. 1), ce qui explique en partie sa rapide propagation. Ces bactéries convertissent l'azote atmosphérique de telle sorte qu'il peut être utilisé par les plantes comme substance nutritive, pratiquement comme engrais. La fixation de l'azote met en outre davantage d'azote à la disposition de l'aulne vert qu'il ne peut en assimiler durablement. Il en résulte que d'autres plantes du sous-bois sont fertilisées.

Tout écosystème est profondément modifié par l'accroissement des quantités d'azote. Certaines plantes particulièrement vigoureuses sont ainsi encouragées et évincent de nombreuses autres espèces. C'est ainsi que le sous-bois d'aulnes verts ne présente plus en général que relativement peu d'espèces végétales telles que l'adénostyle à feuilles d'alliaire ou la laitue des Alpes (fig. 2). Si l'aulne vert couvre plus de la moitié d'une parcelle, la diversité végétale diminue d'environ la moitié et les coléoptères, les sauterelles, les papillons diurnes et d'autres insectes se font nettement plus rares. Le tétras lyre trouve moins de nourriture dans les aulnes verts que sur les prairies et ne nidifient plus dans les épaisses aulnaies vertes.



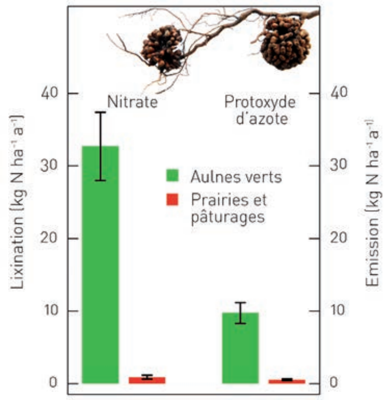


Fig. 1: Lixiviation du nitrate et émission de protoxyde d'azote sont nettement plus élevées dans les aulnaies vertes que dans les prairies (Moyenne ± SE). La fixation de l'azote s'effectue dans les nodosités racinaires.

Les petites aulnaies vertes peuvent tout à fait contribuer à la diversité d'un paysage. Cependant, si l'aulne vert est présent sur de vastes étendues en tant que quasi-monoculture, la diversité écosystémique se réduit d'autant. Comme les prés et pâturages riches en espèces sont nettement plus fréquents dans les zones alpines qu'en plaine, l'appauvrissement de la biodiversité y est considérable.

Pollution du sol, des eaux et du climat

L'excédent d'azote est libéré par divers canaux à partir des peuplements d'aulnes verts ; deux canaux posent particulièrement problème : d'une part, il est lixivié sous forme de nitrate ; d'autre part, il parvient dans l'atmosphère sous forme de protoxyde d'azote (fig. 1).

Entre 15 et 30 kg d'azote nitrique par hectare et par an sont ainsi lixiviés. En même temps, d'autres nutriments importants tels que des cations basiques disparaissent également. Par rapport à une aulnaie verte, une prairie ou une forêt de montagne ne produit pratiquement pas de nitrate. A la suite de l'augmentation d'azote, de nombreux processus accomplis dans le sol (nitrification, assimilation de l'ammonium par les plantes) ont une action acidifiante. Par conséquent, le sol des aulnaies vertes s'acidifie de plus en plus.

Les premières mesures de protoxyde d'azote dans les peuplements d'aulnes verts révèlent des taux d'émission 35 fois supérieurs à ceux observés dans les prairies. Etant donné sa durée de résidence dans l'atmosphère (114 ans en moyenne), le protoxyde d'azote est un gaz à effet de serre presque 300 fois plus fort que le gaz carbonique. Les émissions annuelles de protoxyde d'azote issues d'un hectare d'aulnaie verte sont aussi nocives pour le climat que les émanations de CO_2 de 19 000 kilomètres parcourus en voiture.

Pas de retour de la forêt

Il serait souhaitable que la forêt reconquière les pâturages et prairies de fauche qui ne peuvent être conservées. Une forêt buissonnante peut aussi générer une forêt au fil des ans. Ce n'est pas le cas de l'aulnaie verte. Les graines de conifères ne peuvent germer dans un peuplement d'aulnes verts ou dans son sous-bois. Même la coupe des aulnes verts ne sert pas à grand-chose, car ils repoussent en permanence, et l'offre en azote du sol produit constamment une strate herbacée luxuriante. Faute d'entretien pendant plusieurs décennies, aucune forêt ne peut revenir dans ces conditions. De plus, l'aulne vert ne peut offrir de protection contre l'érosion et les avalanches. En cas d'inondation, il favorise même le glissement des précieuses couches supérieures du sol.

Conséquences économiques

Atteinte au paysage

Le paysage de la Suisse est une ressource naturelle qui possède une valeur actuelle nette considérable. Pour le seul secteur touristique suisse, principal employeur de l'arc alpin, la valeur du paysage est estimée à plus de 68 milliards de francs. La prolifération de l'aulne vert bouleverse le paysage. Par rapport aux paysages de montagne ouverts et riches en microstructures, les touristes et les autochtones considèrent les aulnaies vertes vastes, monotones et impénétrables comme moins belles et moins précieuses. L'embroussaillement provoque ainsi une dépréciation du paysage. Dans le contexte du changement climatique, le paysage rural alpin peut regagner de la valeur en tant que surface de substitution par rapport aux surfaces sèches de la plaine. Une fois embroussaillées, ces surfaces sont difficiles à reconstituer et limitent par conséquent la marge de manœuvre des futures générations. De plus, les aulnaies vertes évaporent plus d'eau que des pâturages entretenus, ce qui réduit l'écoulement et donc, dans une mesure certes minime, la production potentielle d'électricité en été (résultats du projet du Fonds national VALUrsern, achevé en 2012).

Fig. 2: L'épais sous-bois de l'aulne vert empêche la lumière d'atteindre le sol.

MOUTON D'ENGADINE

Le mouton d'Engadine n'est pas seulement précieux dans la lutte contre l'embroussaillement en montagne, mais il présente aussi des propriétés intéressantes du point de vue économique. La race est particulièrement robuste et sa viande est d'excellente qualité. Parmi les races de moutons suisses, elle est championne pour ce qui est du nombre d'agneaux produits par an. Tous ces facteurs devraient plus que compenser son poids d'abattage légèrement inférieur.

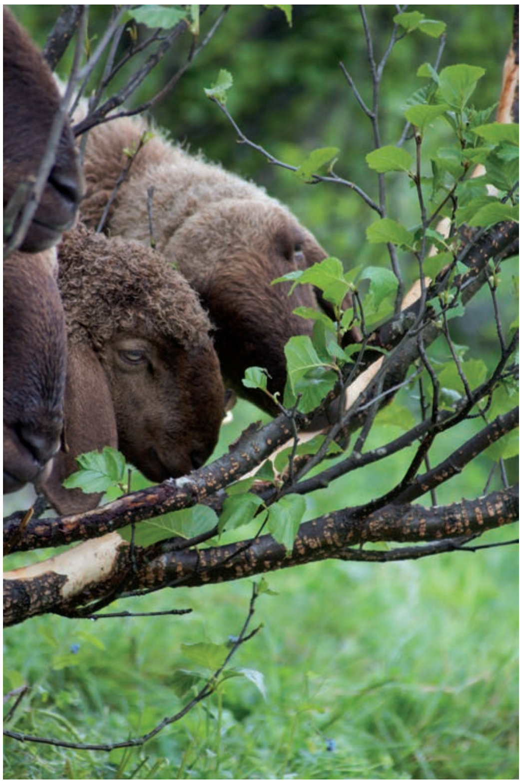


Fig. 3: Le mouton d'Engadine s'attaque à l'écorce de l'aulne vert.

Possibilités d'intervention

Cadre légal

L'article 104 de la Constitution fédérale engage la Suisse « à la conservation des ressources naturelles et à l'entretien du paysage rural ». La politique agricole 2014-2017 entend davantage en tenir compte et prévoit des incitations afin de maintenir le paysage rural de la zone d'estivage. Une étude d'Agroscope a toutefois abouti à la conclusion que les mesures envisagées ne suffiront pas pour enrayer le reboisement et l'embroussaillement des pâturages. La sauvegarde de la biodiversité alpine impose que des mesures soient adoptées à l'échelle nationale et que les surfaces riches en espèces fassent l'objet d'une protection particulière. De même, la population locale accorde une grande importance à ce que l'embroussaillement des terres cultivées situées en altitude soit enrayé.

Mesures de maintien

Le paysage rural ne peut être maintenu que par son utilisation. Les mesures mécaniques (recépage, paillage) sont coûteuses et souvent difficiles sur les parcelles en pente ; elles n'offrent pas de solution durable. Les chèvres et les moutons d'Engadine, en revanche, ont la faculté de manger les pousses et l'écorce de buissons tels que l'aulne vert, ce qui permet leur élimination sans rejets de souche (fig. 3). Après une seule saison de pâturage, les résultats sont déjà visibles. En particulier les surfaces où la pousse ne fait que débuter peuvent être maintenues dégagées grâce à un pacage ciblé. D'autres races de mouton, par contre, ne mangent que l'herbe et évite les écorces des bosquets. Les chèvres et les moutons d'Engadine devraient être encouragés notamment en zone d'estivage, car ils rendent de précieux services en ce qui concerne la lutte contre l'embroussaillement des zones herbagères et la sauvegarde de la biodiversité.

Impressum

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Chapter 4

Shrub expansion of *Alnus viridis* drives former montane grassland into nitrogen saturation

Tobias Bühlmann, Christian Körner, Erika Hiltbrunner

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Abstract

The N_2-fixing shrub *Alnus viridis* is currently encroaching montane grasslands in the Alps as a result of reduced land management and complete abandonment. *Alnus* introduces large amounts of nitrogen (N) into these formerly N-poor grasslands and restricts the succession to montane forests. We studied pools and fluxes of N and the associated C pools in pastures (controls) and adjacent *Alnus* shrublands at two elevations (1650 versus 1950 m a.s.l.) in three valleys in the Swiss central Alps. The total N and C pools stored in 50-year-old *Alnus* shrubland did not exceed those in adjacent pastures with a total of c. 610 g N m^{-2} in phytomass plus soil (down to 30 cm) at both elevations. In *Alnus* stands, reduced soil N pools balanced the gain in phytomass N pools, a likely result of a faster turnover of soil N. The soil solution under *Alnus* was continuously enriched with nitrate, with a total N leaching of 0.79 g N m^{-2} $season^{-1}$ (June to October) under 50-year-old stands at both elevations and the highest flux of 1.76 g N m^{-2} $season^{-1}$ in 25-year-old shrubland at low elevation, clearly indicating an excess of available N in *Alnus* shrubland. In contrast, N leaching across all pastures was close to zero (0.08 g N m^{-2}) throughout the season. At the catchment scale, streamlet water showed increased nitrate concentrations with typical flushing peaks in spring and autumn, provided more than one fifth of the catchment area was covered by *Alnus* shrubs. We conclude that the expansion of *Alnus* rapidly converts centuries-old, N-poor grassland into N saturated shrubland, irrespective of elevation, and it reduces the C storage potential of the landscape since the *Alnus* dominance constrains the re-establishment of a natural montane forest.

1. Introduction

High elevation ecosystems respond sensitively to atmospheric nitrogen (N) deposition, because low temperatures limit soil development, nutrient cycling and plant productivity (Bowman and Seastedt, 2001; Körner, 2003). Even comparatively minor atmospheric inputs of reactive N in the range of 0.5 to 1.0 g N m^{-2} yr^{-1} affect plant community composition and increase plant productivity in alpine and subalpine grassland (Bobbink et al., 2010; Bassin et al., 2013; Roth et al., 2013). N deposition alters the competitive relationships among plant species in favour of nitrophilous species, in particular graminoids, and slow growing taxa typically disappear, leading to reduced plant diversity and evenness (Bobbink et al., 2010; Payne et al., 2013). Presently, critical loads (CL) of N for alpine/subalpine grassland are set to 0.5–1.0 g N m^{-2} yr^{-1} and slightly higher for montane hay meadows to 1.0–2.0 g N m^{-2} yr^{-1} (Bobbink et al., 2011). Critical loads define the threshold of atmospheric N deposition below which undesired effects on sensitive biota are not expected to occur (Nilsson and Grennfelt, 1988).

Since the 1950s and in concert with other global change drivers (e.g., climate warming, land use change), increased atmospheric N deposition has widely been recognized as a threat to biodiversity in terrestrial ecosystems (Stevens et al., 2010; Sutton, 2011). In contrast, the expansion of N_2-fixing plants has rarely been studied in the context of a surplus in N input, although it can introduce large amounts of reactive N into ecosystems (Hiltbrunner et al., 2014). N_2 fixation rates of 0.2–3.6 g N m^{-2} yr^{-1} have been suggested for several biomes, but when N_2-fixing species become dominant, N inputs may even exceed 10 g N m^{-2} yr^{-1} (Binkley, 1981; Cleveland et al., 1999). Irrespective of their origin, high loads of reactive N cause a decline in biodiversity, increases in soil acidification, nitrate leaching and gaseous N losses (Sutton, 2011 and citations therein).

Species-rich, traditionally and sustainably used grassland represents an endangered vegetation type of high conservation value across Europe (Klötzli et al., 2010). Many of these grasslands are confined to the Alps and other mountainous regions (Rudmann-Maurer et al., 2008; Homburger and Hofer, 2012). This type of grassland is either under pressure because of agricultural intensification and urban sprawl (Monteiro et al., 2011), or through reduced grazing and mowing and complete land abandonment, commonly followed by the colonization by woody taxa in the montane belt (MacDonald et al., 2000; Gellrich and Zimmermann, 2007).

In central Europe, *Alnus viridis* [Chaix] DC (= *Alnus alnobetula*, green alder) is currently the most expanding shrub species in terms of land cover, rapidly encroaching former montane pastures and meadows in the Austrian, French, Italian and Swiss Alps (Richard, 1990; Anthelme et al., 2007) and in the Balkan and the Carpathian mountains (Boscutti et al., 2013). The current increase in *Alnus* cover across the Alps is much faster than the re-growth of the montane forest. For instance, in the Swiss Alps, the area covered by so-called tall shrubs (most of this *Alnus*) increased by 35.5% (+11,700 ha) between the 1993–95 and 2004–06 Swiss forest inventories, the forest area by 6.7% (+24,500 ha) within the same period, leading to an overall, combined increase of the area of shrubland and forest of 9.1% (Brändli, 2010). *Alnus viridis* contributes 70% to the total shrub cover in Switzerland (c. 50,000 ha *Alnus* dominated shrubland in 2004/06). Primary production in *A. viridis* is higher than in most other tall shrub species in the montane temperate and Holarctic zones owing to its high N_2-fixing capacity, clonal growth and reproductive success (Körner and Hilscher, 1978; Wiedmer and Senn-Irlet, 2006). *Alnus* rapidly colonizes abandoned grassland, covering hundreds of hectares with nearly monospecific canopy after a few decades. For instance, *Alnus* shrubland increased by 250–300 ha in the Ursern Valley between1994 and 2004 (Thijs van den Bergh, unpublished). When N_2-fixing *Alnus viridis* invades montane grassland this has two major consequences: (1) high amounts of N become available in ecosystems known for their low nutrient status and relatively low atmospheric N inputs, and (2) species-rich grassland of high conservation value becomes replaced by a species-poor, dense shrubland.

Alnus is a globally widespread genus (Hiltbrunner et al., 2014). *A. viridis* represents an aggregate of subspecies found across the Holarctic with centres in Alaska, Northwest-Canada, Northern Europe, Siberia and in the European Alps (David, 2010; Boscutti et al., 2013; Hiltbrunner et al., 2014 and citations therein). The *Alnus* shrub is a typical pioneer plant, forming 2–4 m tall canopies, naturally occurring in disturbed habitats such as avalanche tracks, wet ravines or edges of small, steep creeks. *Alnus* lives in symbiosis with the N_2-fixing actinomycete *Frankia alni*. *Alnus viridis* is commonly unable to spread into established montane forests (Bischof, 1984) and it forms dense thickets between 900 m a.s.l. and the alpine treeline (>2,000 m a.s.l.; Richard, 1967; Körner, 2012). Precise N_2 fixation rates for *A. viridis* are not available. Rehder (1970) estimated rates up to 25 g N m^{-2} yr^{-1}, which we regard as unlikely. Fixation rates of 2.0 up to 6.2 g N m^{-2} yr^{-1} have been reported for North American *Alnus viridis* ssp. *sinuata* and *Alnus viridis* ssp. *crispa* (Binkley, 1981). Owing to their similar habitat preferences and growth form, we assume the N_2 fixation rates to be in the same range for the European *A. viridis* species. On top of these potentially massive N inputs by *A. viridis*, current modelled atmospheric N deposition in the range of 1.0 to 1.5 g N m^{-2} yr^{-1} brings the total N load to a range of 3.0–7.7 g N m^{-2} yr^{-1} in these montane ecosystems (Bühlmann et al., 2015).

In the present study, we assessed *Alnus* induced shifts in fluxes and pools of N and associated changes in soil characteristics such as base saturation, pH and C pools. Carbon pools in *Alnus* were compared with literature data for montane forests to rate the C sequestration potential in *Alnus.* We monitored total N concentrations in the soil solution at a high temporal resolution, nitrate leaching beneath the main rooting horizons and N export to streamlets in micro-catchments. We employed a comparative approach: pairing pastures and adjacent *Alnus* shrublands at two different elevations in three valleys in the Swiss central Alps.

2. Material and methods

2.1. Study sites

The study was carried out along a west-east gradient in three montane valleys (Nufenen, Ursern, Oberalp) in the San Gottardo massif of the Swiss Alps. The Nufenen sites were located in the central orthogneiss zone with mesozoic and penninic sediments, yielding variable soil acidity (Labhart, 2009). The sites in the Oberalp and Ursern Valleys were situated in the northern paragneiss zone with clearly acidic soils. The annual precipitation across the three valleys varies between 1100 mm and 1550 mm (data provided by MeteoSwiss), with high rainfall during summer and a seven- to eight-month dormant season with a heavy snowpack. The long-term annual mean air temperature (1981 to 2010) has been 3.7 °C at the closest weather station of the Nufenen Valley (Ulrichen, 1346 m a.s.l), 4.2 °C in the Ursern Valley (Andermatt, 1438 m a.s.l.) and 6.7 °C in the Oberalp Valley (Disentis, 1197 m a.s.l). In all three valleys the warmest month is July with monthly mean air temperatures ranging between 13.1 and 15.5 °C and the coldest month is January (-7.6 to -1.4 °C).

Table 4.1: Location of the six study sites (consisting of grazed pasture and *Alnus* shrubland at each site, along W–E gradient).

	Low-elevation sites			High-elevation sites		
	Nufenen	Ursern	Oberalp	Nufenen	Ursern	Oberalp
Location	46°29'52" N	46°36'49" N	46°39'11" N	46°28'20" N	46°35'21" N	46°38'31" N
	8°19'37" E	8°34'02" E	8°42'16" E	8°22'24" E	8°29'06" E	8°41'05" E
Elevation (m a.s.l)	1610	1670	1650	2030	1920	1930
Alnus age (years)	c. 40	c. 60*	c. 30	c. 70	c. 50	c. 50

*Additional young (<25 years, at 1530 m a.s.l.) and old (>100 years, at 1590 m a.s.l) *Alnus* stands for suction cups installation (see N in soil solution)

In each valley, c. 50-year-old *Alnus* shrubland was selected on north-exposed slopes at low (c. 1650 m a.s.l.) and high elevation (c. 1950 m a.s.l) with adjacent, still grazed pastures as controls, in <50 m distance to *Alnus* shrubland (Table 4.1). Due to the close proximity, similar topography, and exposure of shrublands and pastures, we assumed similar soil conditions before the *Alnus* encroachment took place. To test for stand age effects, we additionally selected a young (<25 years) and an old (>100 years) *Alnus* stand at low elevation in the Ursern Valley. At all sites, *Alnus* formed dense canopies with an average contribution of *Sorbus aucuparia* L. and *Salix* spp. of <10% cover and an understorey dominated by lush, tall herbs, interspersed with dwarf shrubs (mainly *Rhododendron* and *Vaccinium* species). Based on the thickest stems' age, the *Alnus* shrubs were 30 to 70 years old, corresponding to a mean stand age of c. 50 years. Because of earlier land abandonment, the *Alnus* stands were on average one decade older at the high elevation sites.

2.2. Meteorological measurements and atmospheric bulk N deposition

To delineate microclimatic effects of the *Alnus* expansion, soil temperature was monitored hourly at 10 cm soil depth in pastures and *Alnus* shrubland (one logger per site, 12 in total; Tidbit v2 Temp logger, Onset Computer Corp., USA). Precipitation was measured with a tipping gauge (RAINEW 111, RainWise Inc., USA) positioned 0.4 m above ground in the pastures. Throughfall below the *Alnus* canopy was captured at each site by a 6 m long rain gutter (area: 0.6 m^2; supported by poles at 0.3 m above ground) fitted to a tipping gauge. To assess atmospheric bulk N deposition (i.e., wet and parts of dry deposited N), we connected PET bottles to the tipping gauges to sample rainwater in pastures and throughfall under *Alnus* shrubland for the analysis of dissolved inorganic N (DIN) every two weeks from mid June through the end of September in 2011 and 2012. Samples were immediately frozen and stored at -18 °C. DIN in snow pack was determined in three complete snow profiles per site in the Ursern Valley in April 2012 and April 2013.

2.3. N and C pools in plant material and soil

In order to quantify N and C pools, we determined above- and belowground (to a depth of 30 cm) phytomass (i.e., biomass plus attached necromass) in pastures and *Alnus* stands at all sites at peak biomass (late July and early August) in 2011. In pastures, total above- and belowground plant material was harvested in five 25 x 25 cm quadrats per site. Given the taller plant size and greater patchiness of the aboveground phytomass of understorey plants and total belowground phytomass in *Alnus* stands, both were harvested in five 50 x 50 cm quadrats per site. Aboveground phytomass of pastures and understorey was sorted into graminoids, herbs, dwarf shrubs (leaves and stems separated), ferns and mosses. Belowground plant material was separated into fine roots (<2 mm), woody roots (>2 mm), non-woody roots (>2 mm), rhizomes and *Alnus* root nodules.

We collected leaves and branches of five *Alnus* shrubs within each site. Branches were separated into xylem and phloem. Further, we measured the diameter of all *Alnus, Sorbus* and *Salix* stems in two 2 x 20 m transects. We then calculated wood and leaf dry matter per unit land area using allometric relationships between stem diameter ('diam') and wood or leaf dry matter (equations 1–4; van den Bergh et al., unpublished). Leaf area index (LAI, $m^2\ m^{-2}$) of the canopy was calculated by converting total leaf dry matter to total leaf area using the specific leaf area (SLA, $m^2\ kg^{-1}$).

Alnus and *Sorbus* (n = 33 *Alnus* stems; n = 32 *Sorbus* stems)

Leaf mass (dry weight, g) = $\text{diam}^{2.19}$ (mm) x $10^{-1.38}$ ($R^2 = 0.96$, $p < 0.001$) eq. 1

Wood mass (dry weight, g) = $\text{diam}^{2.75}$ (mm) x $10^{-1.27}$ ($R^2 = 0.99$, $p < 0.001$) eq. 2

Salix ssp. (n = 12 stems)

Leaf mass (dry weight, g) = $\text{diam}^{2.51}$ (mm) x $10^{-2.03}$ ($R^2 = 0.98$, $p < 0.001$) eq. 3

Wood mass (dry weight, g) = $\text{diam}^{2.50}$ (mm) x $10^{-0.93}$ ($R^2 = 0.99$, $p < 0.001$) eq. 4

At each site, fresh-fallen litter of *Alnus* was collected from three litter traps (area: 0.2 m^2) every two weeks during the growing season until late autumn in 2012. Litter of pastures and understorey plants was manually collected in three 25 x 25 and 50 x 50 cm quadrats per site respectively, in October 2012.

To determine soil N and C pools of the main rooting horizon, soil cores were taken from 0–5, 5–10, 10–20 and 20–30 cm depth and sieved (2 mm) for further analysis (n = 5 per site and soil depth). Plant and soil material was dried at 80 °C before weighing.

2.4. Chemical analysis

Representative subsamples of all tissue types, litter and soil were ground to powder and analysed for N and C concentration by an elemental analyser (FlashEA 1112, ThermoFinnigan, Italy). Furthermore, $\delta^{15}N$ values were analysed in various *Alnus* tissues (leaves, branch phloem, branch xylem, roots and nodules). In *Alnus*, we pooled samples to one sample per tissue type and site, except for foliage, where we collected leaves from five shrubs per site. In order to demonstrate how non-N_2-fixing plants in the proximity of *Alnus* were affected by its N_2 fixation, foliar $\delta^{15}N$ content was examined in *Solidago virgaurea* L. (Asteraceae), *Rhododendron ferrugineum* L. and *Vaccinium myrtillus* L. (both Ericaceae; n = 5 leaf samples of 5 individuals per species and site), occurring in both pastures and in the understorey of *Alnus* stands. $\delta^{15}N$ was analysed by an isotope ratio mass spectrometer (Delta-S Finnigan MAT, Germany).

We measured pH of soil samples in 0.01 M $CaCl_2$ solution. Actual base saturation (ratio of the exchangeable base cations Mg^{2+}, Ca^{2+}, K^+ and Na^+ to the actual cation-exchange capacity, i.e., sum of all exchangable cations including Al^{3+} and H^+, see Blume et al., 2010) was analysed in three soil profiles of each pasture and shrubland site for each soil layer separately by adding 50 ml of 0.5 M NH_4Cl to 2.5 g dried, sieved soil. Na^+ and K^+ were measured by flame emission with air/C_2H_2 and Ca^{2+}, Mg^{2+} and Al^{3+} by atomic absorption photometry with N_2O/C_2H_2 (Varian AA240FS, Aligent, USA). Total acidity of the NH_4Cl-extracts was titrated with 0.01 M NaOH.

2.5. N in soil solution and N leaching

To monitor N concentration of the soil solution, ceramic suction cups (SK20, UMS GmbH, Germany) were positioned beneath the main rooting horizon at a soil depth of 40 cm with an angle of 45° to the soil surface in pastures and *Alnus* shrubland in the Ursern Valley at low and high elevation and additionally, in young and old *Alnus* stands (Table 4.1). Each suction cup was connected to a bottle set under vacuum (initially -0.55 bar, not kept constant). Soil solution of the first three weeks after installation was discarded, then, sampled weekly throughout the season until the end of October, i.e., as long as snow depth permitted accessing the probes. In 2010, samples were collected at low elevation sites only. In 2011, and from June to July in 2012 samples were obtained from both elevations. In winter 2011/2012 (Nov, Dec and Jan), a subset of suction cups was located under deep snow at low elevation. All samples were frozen and stored at -18 °C.

In addition to suction cups, zero-tension lysimeters (ZTL) were installed to quantify N leaching rates below the main rooting horizon. These devices were built by cutting stainless steel pipes (30 cm long, 6 cm in diameter) in half over a length of 18.5 cm. At the uncut end, each pipe was sealed by a lid (with an air filter disk attached) and connected by a plastic tube to a 1.5 L PET bottle wrapped in aluminium foil. We inserted these pipes through the upslope wall of ditches (c. 35 cm deep and 30 cm wide) to allow a position parallel to the slope under undisturbed profiles at a soil depth of 30 cm. At each site, eight zero-tension lysimeters were installed in pastures and eight in *Alnus* shrubland nine months prior to first sampling (96 ZTLs in total). Soil solution was then collected every two weeks from the beginning of June through the end of October 2012.

The amounts of water collected by ZTLs and suction cups neither corresponded to the amount of seepage water calculated by the ecosystem water balance (see below), nor were they correlated with the N concentration in the soil solution. To estimate nitrate-N and ammonium-N leaching per unit land area, nitrate-N and ammonium-N concentrations were multiplied by the amount of seeped water as derived from a simplified water balance equation. Since there was no significant surface runoff at these sites (Alaoui et al., 2014), seepage resulted from subtracting evapotranspiration (ET) from measured precipitation for spring (1st June to 30th June), summer (1st July to 31th August) and autumn (1st September to 24th of October). We calculated monthly ET from data published in the Hydrological Atlas of Switzerland (Menzel et al., 2001) by multiplying annual ET for each site by the fraction each month contributes to the annual total (Baumgartner et al., 1983). Changes in soil water storage were assumed to be very small because soils remained wet over the entire season (repeated resetting to field capacity by rain events).

2.6. N export into micro-catchments

In the Ursern Valley, we also assessed the influence of increasing *Alnus* cover at the landscape scale. We measured the N concentration in streamlet water in four micro-catchments (<1 km^2) located on north-east and north-west facing slopes (Table 4.2). Each streamlet was equipped with an autosampler (Isco 2700, Teledyne Isco, USA), a gauging station and a weather station at the outflow of the catchment (for details see: S. Lagger, MSc Thesis University of Bern, 2012). Water samples were collected daily (at 2 p.m.) during the growing season until the end of October in 2011. In winter, one sample per month was taken manually. Samples were kept frozen until the analysis for DIN.

Table 4.2: Characterisation of the four micro-catchments in the Ursern valley (ordered by increasing *Alnus* cover).

	Laubgädem	Wallenboden	Bonegg	Chämleten
Projected area (ha)[a]	3.0–11.1	38.4–60.1	34.3–71.3	1.9–2.0
Alnus cover (%)[a]	4–14	13–23	22–38	82–89
Mean discharge 2011 (L s^{-1})[b]	3.2 ± 0.1	2.4 ± 0.1	6.2 ± 0.2	1.1 ± 0.1

[a] Combined data of Mueller et al. (2013), Lagger (MSc thesis University of Bern, 2012) and T. van den Bergh (pers. communication). Since the exact area and thus the shrub cover of micro-catchments were difficult to determine, we here present the range estimated by the different authors

[b] data provided by S. Lagger

2.7. Analysis of DIN and DON concentration in water and soil solution samples

The concentrations of DIN (NO_2^-, NO_3^- and NH_4^+) in rain (from bulk sampler), throughfall, snow, soil solution and streamlet water samples were measured by means of two ion chromatographs (Dionex ICS 900, Thermo Scientific, USA; detection limit for NO_3^--N: 0.05 mg N L^{-1}; for NO_2^--N and NH_4^+-N: 0.01 mg N L^{-1}). DON was indirectly analysed by subtracting DIN from the total nitrogen bound (TNb) measured by a TOC/TNb analyser (DIMA–TOC 100, Dimatec Analysentechnik GmbH, Germany; detection limit: 1 mg TNb L^{-1}). DON was measured in selected subsets of samples only: for soil solution, in samples collected before (mid August 2010) and after leaf fall (beginning of November 2010) as well as in streamlet water in two 6-day series before and after leaf fall in 2011.

2.8. Statistics

Statistical analyses were performed using the software R (version 3.1.1; R Development Core Team, 2013). The effects of vegetation type (i.e., pasture versus *Alnus*) on the different dependent variables (total phytomass, total N and C pools, $\delta^{15}N$, N concentration of plant tissues, pH($CaCl_2$), base saturation, N leaching) were tested using linear mixed effect models of the package 'nlme' (Pinheiro et al., 2014). The effect of the different valleys was treated as random factor and elevation was nested within each valley. In case of non-normality or inhomogeneity of the data, we applied log transformations to the response variables and corrected to the needed variance structure. Analyses of suction cup data were restricted to means per suction cup and season, and for N leaching to sums per site. We accounted for the variation within sites when the standard error per site was calculated (error propagation).

3. Results

3.1. Site conditions

Overall, the annual mean soil temperature was 5.1 °C in pastures and 4.5 °C under *Alnus* at low elevation, and 4.1 °C in pastures and 3.5 °C under *Alnus* at the high sites (Table 4.3). During the growing season, soil temperature under pastures was on average 12.3 °C and 11.3 °C at low and high elevation, and soils were generally 2.4 K cooler under *Alnus* at both elevations. On average, snow cover lasted 180 days at the low and 214 days at the high sites. During the growing season, precipitation ranged between 400 mm at the low sites and 500 mm at the high sites. Across sites and years, bulk atmospheric N deposition accumulated to 0.18 g N m^{-2} at low and 0.29 g N m^{-2} at high elevation from June to September. By the end of winter, snow pack stored 0.06 g N m^{-2} at the low and 0.12 g N m^{-2} at the high sites. Canopy throughfall of *Alnus* contained 0.06 g N m^{-2} at low and 0.10 g N m^{-2} at high elevation during the growing season, indicating an uptake of N by the *Alnus* canopy. LAI of *Alnus* was on average 3.6 m^2 m^{-2} and did not significantly change with elevation. Soil bulk density varied a lot between sites (0.43–0.64 g cm^{-3}) but was similar in *Alnus* stands and adjacent pastures at both elevations.

Table 4.3: Mean soil temperature at 10 cm soil depth (absolute minimum and maximum in parentheses), meteorological characteristics, bulk atmospheric N deposition, LAI of *Alnus* stands and soil bulk density of the study sites (mean ± se).

	Low-elevation sites			High-elevation sites		
	Nufenen	Ursern	Oberalp	Nufenen	Ursern	Oberalp
T_{soil} annually, pasture (°C)[a]	4.7 (0.0, 15.7)	5.2 (0.3, 14.2)	5.4 (-0.9, 19.3)	3.4 (-0.3, 19.8)	4.7 (0.4, 14.8)	4.2 (-0.2, 14.8)
T_{soil} growing season, pasture (°C)[b]	11.6 (5.9, 15.4)	11.4 (7.2, 14.3)	13.7 (6.4, 19.3)	11.7 (1.9, 19.6)	11.3 (6.8, 14.1)	11.0 (5.7, 14.1)
T_{soil} annually, *Alnus* (°C)[a]	4.3 (0.2, 13.2)	4.3 (0.6, 12.3)	4.8 (0.3, 12.8)	2.6 (-0.4, 13.0)	4.9 (0.8, 15.8)	3.0 (0.0, 11.3)
T_{soil} growing season, *Alnus* (°C)[b]	9.9 (5.0, 13.1)	9.5 (5.7, 12.2)	10.5 (7.0, 13.1)	7.5 (2.5, 12.8)	10.7 (4.9, 15.9)	8.3 (4.2, 11.2)
Snow cover (days)[c]	184	176	179	245	186	212
1st snow-free day in 2012	May, 13th	May, 10th	May, 7th	June, 8th	May, 23th	June, 6th
Precipitation (mm growing season^{-1})[d]	355[e]	396 ± 33	435 ± 5	560 ± 72	468 ± 72	494 ± 11
Bulk N deposition (g N m^{-2} season^{-1})[d]	0.16 ± 0.08	0.23 ± 0.05	0.15 ± 0.02	0.23 ± 0.07	0.43[f]	0.20 ± 0.07
N in snow pack (g N m^{-2})[e]	-	0.06 ± 0.03	-	-	0.12 ± 0.02	-
Alnus LAI (m^2 m^{-2})	4.5 ± 0.4	3.4 ± 0.3	3.3 ± 0.2	3.3 ± 0.7	3.7 ± 1.6	3.3 ± 0.5
Soil bulk density *Alnus* (g cm^{-3})	0.43 ± 0.02	0.55 ± 0.03	0.64 ± 0.03	0.54 ± 0.03	0.51 ± 0.02	0.51 ± 0.03
Soil bulk density pastures (g cm^{-3})	0.43 ± 0.06	0.55 ± 0.03	0.60 ± 0.04	0.58 ± 0.02	0.54 ± 0.03	0.52 ± 0.04

[a] August to August in 2011/2012 and 2012/2013

[b] mid June to end September in 2012

[c] winter 2012/2013

[d] June to end September in 2011 and 2012

[e] 2012 only

[f] 2011 only

3.2. Phytomass, nitrogen and carbon concentrations and pools

The total dry weight of phytomass (above- plus belowground) on pastures was 1890 g m^{-2} at low and 1730 g m^{-2} at high elevation. Phytomass was substantially higher in *Alnus* stands (including the understorey), largely due to the accumulated dry matter in *Alnus* stems, yielding 7360 g m^{-2} at low and 5350 g m^{-2} at high elevation, with large scatter between individual sites (Table 4.4). Under both vegetation types, belowground plant material was c. 1560 g m^{-2} at low and c. 1280 g m^{-2} at high elevation. Peak season green phytomass (largely foliage), which represents a proxy for annual aboveground productivity (disregarding perennial mosses), was 560 g m^{-2} at low and 400 g m^{-2} at high sites in shrubland (including the understorey) with lower values for pastures ($F_{1,5} = 5.4$, $p = 0.07$), namely 370 g m^{-2} and 320 g m^{-2} for low and high elevation. Estimated annual wood increment (wood mass divided by stand age) added another 99 ± 19 g m^{-2} (mean ± se) to the annual productivity in shrubland, resulting in a 64% higher annual phytomass production than in pastures.

Leaves of plants growing in the understory of *Alnus* had similar high N concentrations as *Alnus* leaves: 27.3 ± 4.5 mg N g^{-1} (mean ± se) at low and 30.7 ± 3.3 mg N g^{-1} at high elevation (Table 4.4, N concentrations based on dry weight). *Alnus* leaves showed slightly higher N concentration at the higher elevation (29.4 ± 0.4 versus 28.2 ± 0.2 mg N g^{-1} at lower elevation). The mean N concentration in aboveground phytomass in pastures was 19.7 ± 2.8 mg N g^{-1} at the low and 18.9 ± 2.8 mg N g^{-1} at the high sites. Tissue specific N concentrations were scaled to N pools by multiplying N concentrations by dry matter and summed up for above- and belowground compartments and soil (0–30 cm).

Fresh fallen litter contained on average 9 g N m^{-2} in shrubland (including understorey and across both elevation, Table 4.4), of which 86% originated from the *Alnus* canopy. In pastures, N input via autumn litter was c. 1 g N m^{-2} at both elevations, but this amount reflects just a very small fraction of the annual litter production, because leaf turnover continues throughout the growing season and the majority of aboveground litter is produced very late in autumn and winter. Interestingly, the quantity of N in *Alnus* litter was only 10% lower than in green leaves at peak biomass, indicating that *Alnus* resorbed little N from its foliage before leaf shedding.

Table 4.4: Dry weight (g m^{-2}), N concentration (mg N g^{-1} dry weight) and N pools (g m^{-2}) of plant material and soil (0–30 cm, mean ± se) of pastures and *Alnus* stands at low and high elevation.

	Pasture			*Alnus*		
	Dry weight	N conc.	N pool	Dry weight	N conc.	N pool
Low elevation						
Non-*Alnus* aboveground green phytomass						
Grasses	292 ± 103	17.1 ± 1.3	4.5 ± 1.2	9 ± 7	26.8 ± 2.6	0.2 ± 0.1
Herbs	73 ± 16	22.5 ± 1.3	1.7 ± 0.3	120 ± 57	31.6 ± 2.6	3.7 ± 1.9
Dwarf shrubs leaves	3 ± 1	22.3 ± 0.7	0.1 ± 0.0	46 ± 42	22.3 ± 0.2	1.0 ± 0.9
Ferns	-	-	-	68 ± 13	30.2 ± 2.3	2.0 ± 0.2
Mosses	9 ± 2	16.8 ± 2.0	0.2 ± 0.0	11 ± 3	25.6 ± 1.1	0.3 ± 0.1
Total	377 ± 104	19.7 ± 2.8[#]	6.4 ± 1.2	255 ± 72	27.3 ± 4.5[#]	7.1 ± 2.1
Dwarf shrub stems	6 ± 2	14.1 ± 1.1	0.1 ± 0.0	129 ± 117	12.6 ± 0.8	1.4 ± 1.2
Alnus aboveground phytomass[a]						
Leaves	-	-	-	311 ± 29	28.2 ± 0.2	8.4 ± 0.8
Phloem	-	-	-	858 ± 164	11.6 ± 0.1	9.7 ± 2.0
Xylem	-	-	-	4189 ± 801	4.3 ± 0.1	17.3 ± 3.2
Total	-	-	-	5357 ± 818	-	35.4 ± 3.9
Belowground phytomass						
Fine roots (< 2 mm)	1506 ± 279	14.2 ± 0.7	21.3 ± 3.3	251 ± 47	18.5 ± 1.0	4.5 ± 0.7
Roots (> 2 mm, not woody)	-	-	-	34 ± 14	16.7 ± 1.2	0.5 ± 0.2
Roots (> 2 mm, woody)	-	-	-	1216 ± 631	11.2 ± 1.0	12.2 ± 7.6
Rhizomes	-	-	-	102 ± 41	22.3 ± 2.6	2.2 ± 1.2
Nodules	-	-	-	13 ± 3	26.4 ± 1.6	0.3 ± 0.1
Total	1506 ± 279	14.2 ± 0.7	21.3 ± 3.3	1616 ± 634	19.0 ± 3.6[#]	19.6 ± 7.7
Total						
Phytomass	1889 ± 298	-	27.8 ± 3.5	7357 ± 1044	-	63.5 ± 9.0
Litter[b]	39 ± 6	19.3 ± 1.1	0.8 ± 0.1	269 ± 21	33.2 ± 0.9	9.1 ± 0.8
Soil (0–30 cm)	-	6.7 ± 0.6	699.9 ± 182.3	-	3.9 ± 0.5	511.1 ± 141.2
Total (without litter)	-	-	727.6 ± 182.3	-	-	574.7 ± 141.5
High elevation						
Non-*Alnus* aboveground green phytomass						
Grasses	213 ± 56	16.2 ± 1.1	3.5 ± 1.0	6 ± 3	25.2 ± 1.9	0.1 ± 0.1
Herbs	77 ± 16	20.9 ± 1.0	1.6 ± 0.4	81 ± 37	33.1 ± 1.8	2.5 ± 1.2
Dwarf shrubs leaves	26 ± 23	22.6 ± 0.4	0.5 ± 0.5	8 ± 6	24.7 ± 0.9	0.2 ± 0.2
Ferns	-	-	-	15 ± 15	37.1 ± 0.5	0.5 ± 0.5
Mosses	15 ± 11	15.7 ± 2.3	0.3 ± 0.2	1 ± 1	33.3 ± 1.7	0.0 ± 0.0
Total	331 ± 64	18.9 ± 2.8[#]	5.9 ± 1.2	111 ± 40	30.7 ± 3.3[#]	3.4 ± 1.3
Dwarf shrub stems	24 ± 17	12.6 ± 0.4	0.3 ± 0.2	23 ± 18	12.9 ± 1.8	0.2 ± 0.2
Alnus aboveground phytomass[a]						
Leaves	-	-	-	291 ± 13	29.4 ± 0.4	8.4 ± 0.3
Phloem	-	-	-	655 ± 28	12.6 ± 0.4	8.0 ± 0.1
Xylem	-	-	-	3095 ± 175	4.2 ± 0.2	12.5 ± 0.2
Total	-	-	-	4042 ± 178	-	28.9 ± 0.4
Belowground phytomass						
Fine roots (< 2 mm)	1375 ± 366	10.5 ± 1.1	16.1 ± 6.5	271 ± 54	18.6 ± 0.9	5.0 ± 1.0
Roots (> 2 mm, not woody)	-	-	-	65 ± 22	17.3 ± 1.3	1.0 ± 0.2
Roots (> 2 mm, woody)	-	-	-	688 ± 336	10.2 ± 0.5	7.1 ± 4.0
Rhizomes	-	-	-	128 ± 37	23.5 ± 1.7	3.1 ± 1.2
Nodules	-	-	-	23 ± 10	29.3 ± 1.0	0.6 ± 0.3
Total	1375 ± 366	10.5 ± 1.1	16.1 ± 6.5	1175 ± 343	19.8 ± 2.6[#]	16.7 ± 4.3
Total						
Phytomass	1730 ± 372	-	22.2 ± 6.6	5351 ± 389	-	49.3 ± 4.5
Litter[b]	34 ± 8	16.0 ± 1.2	0.5 ± 0.1	269 ± 16	30.3 ± 0.9	8.7 ± 0.6
Soil (0–30 cm)	-	5.0 ± 0.4	669.2 ± 83.2	-	4.6 ± 0.4	604.3 ± 93.3
Total (without litter)	-	-	691.5 ± 83.5	-	-	653.5 ± 93.4

[a] Including *Sorbus* (on average below 6% of the total dry weight) and *Salix* (on average below 1% of the total dry weight)

[b] Canopy litter, including understorey/pasture litter collected manually in October

[#] Total concentration was calculated from means of 'non-*Alnus* aboveground green phytomass' or 'belowground phytomass'

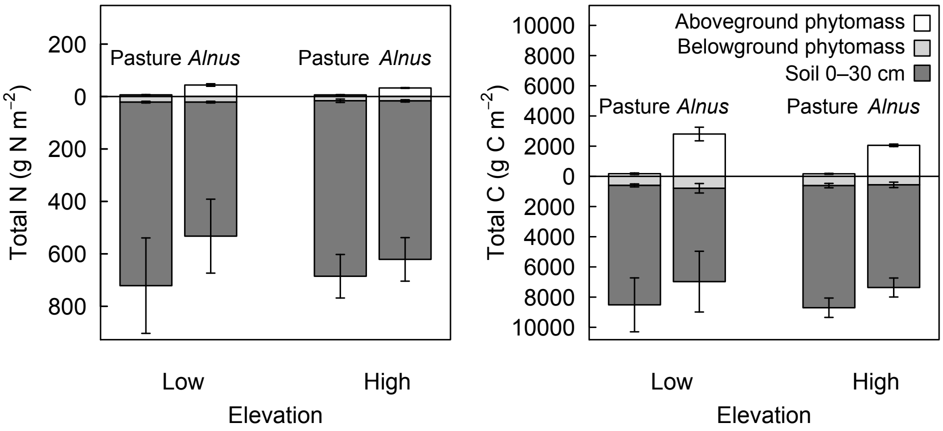

Figure 4.1: Total N and C pools in aboveground phytomass, belowground phytomass and soil (0 to 30 cm; mean ± se) of pastures and *Alnus* shrublands at low and high elevation. Bars above and below the zero line indicate aboveground and belowground pools, respectively.

N pools in the phytomass of *Alnus* stands were 64 g N m^{-2} at low and 49 g N m^{-2} at high elevation (including understorey and belowground phytomass), with N pools of 28 g N m^{-2} and 22 g N m^{-2} in the neighbouring pastures. These significantly higher phytomass N pools in *Alnus* ($F_{1,4} = 115.1$, $p < 0.001$) were largely contained in stems and foliage. In contrast, N pools of belowground phytomass were similar under both vegetation types, namely 20 g N m^{-2} at low and 16 g N m^{-2} at high elevation. Thus, in pastures 74% of all N in phytomass was stored belowground, whereas in *Alnus* 32% was belowground (Fig. 4.1).

The C concentration of different tissues (Table S4.1) scaled to c. 780 g C m^{-2} in phytomass at low and to the same value at high elevation in pastures. In *Alnus* stands, the C pools in phytomass were higher, namely 3590 g C m^{-2} at low and 2620 g C m^{-2} at high sites (Fig. 4.1). As with N, 79% of plant C was stored belowground in pastures, compared to only 22% in *Alnus* stands.

3.2.1. Soils and ecosystem pools of N and C

In soil, both N ($F_{1,52} = 7.6$, $p < 0.01$) and C concentrations ($F_{1,52} = 5.0$, $p < 0.05$) were lower under *Alnus* at low and high elevation compared to pastures. Converting these concentrations into pools per unit land area (0 to 30 cm), the resulting soil N and C stocks were lower under *Alnus*, but variation in soil bulk density, both under *Alnus* and pastures, caused these differences to be statistically not significant (Fig. 4.1). Summing up soil and phytomass pools, there was a trend towards lower total N pools and only slightly increased total C pools in *Alnus* stands compared to adjacent pastures with no effect of elevation. Accounting for the age of shrubland at each site, we arrived at losses of the total N pools in the range of 0.4–10.9 g N m^{-2} yr^{-1} at low and -2.8–4.3 g N m^{-2} yr^{-1} at high elevation in *Alnus* stands since *Alnus* started to overgrow these areas.

3.3. δ^{15}N and N concentration of *Alnus* and understorey plants

As expected, *Alnus* root nodules were significantly enriched in ^{15}N (5.5 ± 0.2‰; mean across both elevations ± se) but stem xylem (-1.4 ± 0.1‰) and phloem (-1.9 ± 0.1‰), leaves (-2.2 ± 0.1‰), and roots (-2.5 ± 0.5‰) had negative δ^{15}N values ($F_{4,\,40} = 637.3$, $p < 0.001$), indicating internal fractionation processes in *Alnus*. These tissue specific $\delta\,^{15}$N values resulted in a whole-plant *Alnus* δ^{15}N value of -1.6 ± 0.1‰ by accounting for the tissue mass fractions. Leaves of the three non-N_2-fixing species that grew under *Alnus* and adjacent pastures revealed enriched δ^{15}N values and higher N concentrations in the presence of *Alnus* (Fig. 4.2; δ^{15}N: $F_{1,\,163} = 7.9$, $p < 0.01$; foliar N concentrations: $F_{1,156} = 128.2$, $p < 0.001$). Leaf δ^{15}N values of these species were slightly more negative at high compared to low elevation, both with and without *Alnus* ($F_{1,2} = 14.4$, $p = 0.06$).

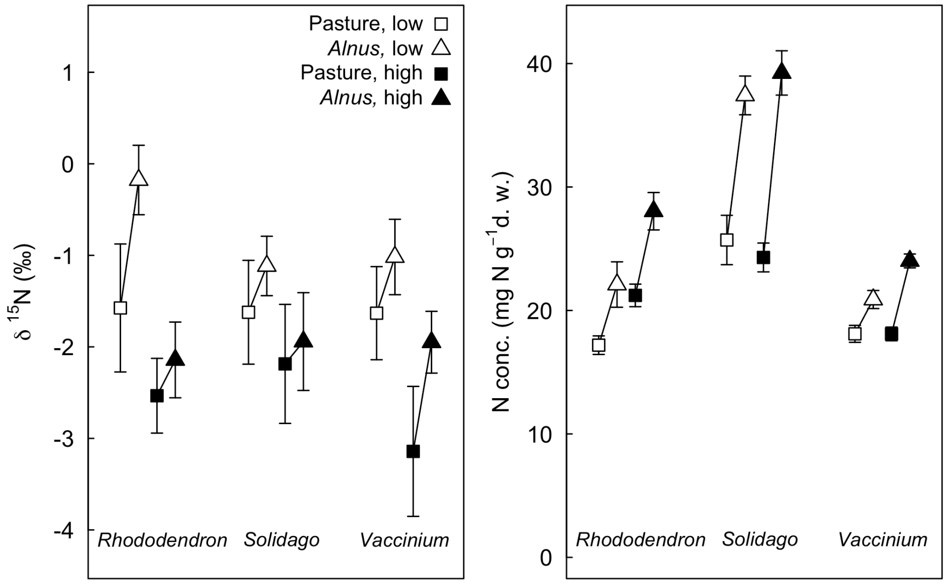

Figure 4.2: Foliar δ^{15}N and N concentration (mean ± se) of the three non-fixing species growing in pastures and adjacent *Alnus* shrubland at low and high elevation (*Solidago* represents a forb; *Rhododendron* and *Vaccinium* are dwarf shrubs).

3.4. *Alnus* induced changes in soils

At a soil depth of 0–30 cm, pH($CaCl_2$) was on average 0.35 units lower under *Alnus* than under adjacent pastures (Fig. 4.3; $F_{1,\,208} = 56.8$, $p < 0.001$). This *Alnus* induced soil acidification was stronger at high than at low elevation (elevation x vegetation type: $F_{1,\,208} = 9.5$, $p < 0.01$), most likely because *Alnus* stands were about one decade older at higher elevation. In *Alnus* stands, soil acidification was more pronounced in the top soil and decreased with soil depth (higher pH). In pastures, soil pH varied little with soil depth (soil depth x vegetation type: $F_{1,\,208} = 16.6$, $p < 0.001$). In line with the pH data, actual base saturation was 15% lower in soils under *Alnus* than in adjacent pastures at low and high elevation ($F_{1,\,127} = 16.0$, $p < 0.001$) and relative differences between pastures and *Alnus* tended to be slightly larger at high compared with low elevation (n. s.).

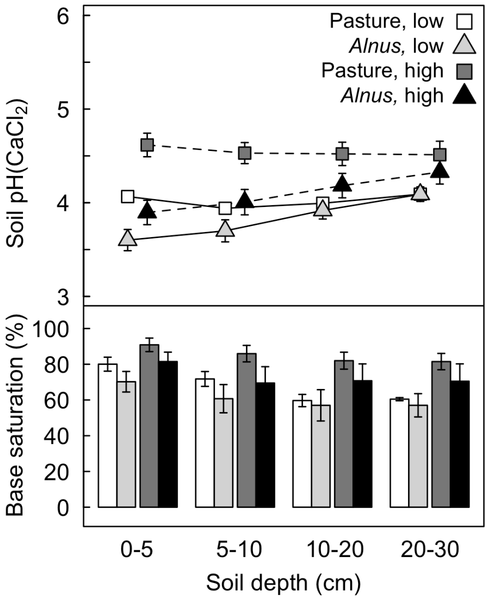

Figure 4.3: Soil pH and actual base saturation (mean ± se) across the soil profile under pastures and *Alnus* shrubland at low and high elevation (note: the same colours were used for the two different vegetation types in the upper and the lower part of the figure).

3.5. N in soil solution and N leaching

In contrast to pastures, the soil solution was highly and continuously enriched in NO_3^--N under *Alnus* (Fig. 4.4; $F_{1,46} = 250.7$, $p < 0.001$) with a mean NO_3^--N concentration of 2.5 ± 0.4 mg NO_3^--N L^{-1} at low elevation (single highest concentration: 15.6 mg NO_3^--N L^{-1}) and of 0.5 ± 0.2 mg NO_3^--N L^{-1} at high elevation (maximum: 4.8 mg NO_3^--N L^{-1}) throughout the sampling period. In pastures, the soil solution commonly contained less than 0.1 mg NO_3^--N L^{-1} (95% of all samples; maximum: 0.5 mg NO_3^--N L^{-1}) and NO_3^--N concentration did not vary with elevation (elevation x vegetation type: $F_{1,46} = 24.9$, $p < 0.001$). At low elevation, the NO_3^--N concentration of the soil solution was twice as high under young (<25 years) *Alnus* compared to 60-year-old stands ($F_{2,26} = 7.1$, $p < 0.05$) but there was no significant difference between 60-year-old and very old (>100 years) stands. At low elevation and irrespective of the stand age, the NO_3^--N concentration decreased on average by 40% at the onset of the growing season but then raised again in autumn during and after leaf fall. Winter NO_3^--N concentrations were very similar to those in autumn, hence, they remained high (data not shown). This seasonal pattern was less pronounced in *Alnus* stands at high elevation and was fully absent in pastures. The concentrations of NH_4^+-N were below 0.2 mg L^{-1} and also NO_2^--N and DON concentrations were always below 0.01 mg NO_2^--N L^{-1} and 1.0 mg DON L^{-1} (analytical detection limits).

Table 4.5: N leaching in pastures and adjacent *Alnus* stands at low and high elevation.

	Low elevation			High elevation	
	Pasture	*Alnus*	*Alnus* (>25 y)	Pasture	*Alnus*
All sites					
Zero-tension lysimeters (Jun–Oct 2012)					
NO_3^- leaching (g NO_3^--N m^{-2})	0.04 ± 0.00	0.49 ± 0.05	-	0.04 ± 0.01	0.78 ± 0.14
NH_4^+ leaching (g NH_4^+-N m^{-2})	0.04 ± 0.00	0.20 ± 0.08	-	0.04 ± 0.01	0.11 ± 0.03
Ursern valley					
Zero tension lysimeters (Jun–Oct 2012)					
NO_3^- leaching (g NO_3^--N m^{-2})	0.04 ± 0.00	0.34 ± 0.06	-	0.01 ± 0.00	1.02 ± 0.34
NH_4^+ leaching (g NH_4^+-N m^{-2})	0.06 ± 0.01	0.33 ± 0.22	-	0.02 ± 0.01	0.05 ± 0.02
Suction cups (Jul–Oct 2010)					
NO_3^- leaching (g NO_3^--N m^{-2})	0.01 ± 0.01	0.91 ± 0.05	1.45 ± 0.14	-	-
NH_4^+ leaching (g NH_4^+-N m^{-2})	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	-	-
Suction cups (Jun–Oct 2011)					
NO_3^- leaching (g NO_3^--N m^{-2})	0.18 ± 0.02	0.82 ± 0.04	1.76 ± 0.18	0.03 ± 0.01	0.26 ± 0.04
NH_4^+ leaching (g NH_4^+-N m^{-2})	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.01	0.04 ± 0.01

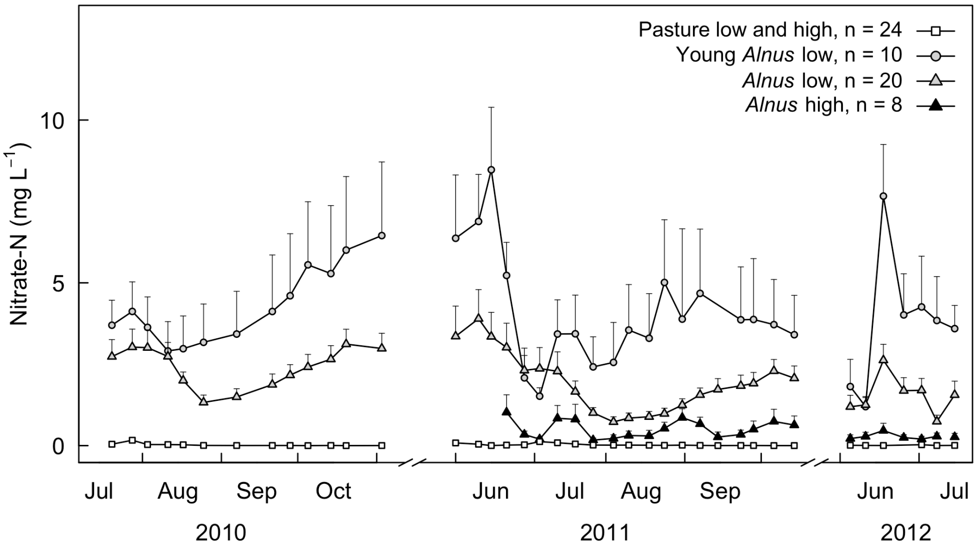

Figure 4.4: Nitrate-N concentration of soil solution in pastures and adjacent *Alnus* at low and high elevation at the core site (mean + 1 se). '*Alnus* low' includes data from the 60-year-old and the more than 100-year-old *Alnus* stands. Note: the year 2010 was not included in statistical analysis.

These very low N concentrations in the soil solution under pastures arrived at a seasonal total leaching of 0.08 ± 0.01 g N m^{-2} at both elevations (June to October 2012; soil water collected by zero-tension lysimeters; Table 4.5). During the same period, N leaching under *Alnus* was tenfold higher ($F_{1,4} = 117.1$, $p < 0.001$) and accumulated to 0.69 ± 0.09 g N m^{-2} at low and 0.89 ± 0.15 g N m^{-2} at high elevation. Nitrate-N contributed 71% and 87% to the total N leaching. The highest leaching of 1.76 ± 0.18 g N m^{-2} season^{-1} was found under 25-year-old *Alnus* from June to October in 2011.

3.6. N in stream flow water

The NO_3^--N concentrations of the streamlet water in the micro-catchment with the lowest *Alnus* cover (4–14%, Laubgädem) were always close to zero (Fig. 4.5). In contrast, the three other micro-catchments with an *Alnus* cover of >20% up to almost 90% revealed higher NO_3^--N concentrations in the streamlet water. In these catchments, NO_3^--N concentrations were lowest during peak season and increased with the onset of *Alnus* leaf fall. The autumnal increase in NO_3^--N was most pronounced (3.1-fold higher compared to peak season) in the catchment with the highest *Alnus* cover (Chämleten). In general, streamlet flow was dominated by base flow, contributing 82–93% to total flow throughout the measuring period. Peak flow was observed only after heavy rainfall events (on eleven days during the growing season 2011). In the catchment with the highest *Alnus* cover, NO_3^--N concentrations of the streamlet water increased from 0.1–0.2 mg NO_3^--N L^{-1} up to 0.5 mg NO_3^--N L^{-1} at peak flow during the growing season and these short-term NO_3^--N peaks further doubled (up to 1.3 mg NO_3^--N L^{-1}) with and after the onset of leaf fall. In the catchments with lower *Alnus* cover (Bonegg, Wallenboden) such temporal shifts were usually less pronounced.

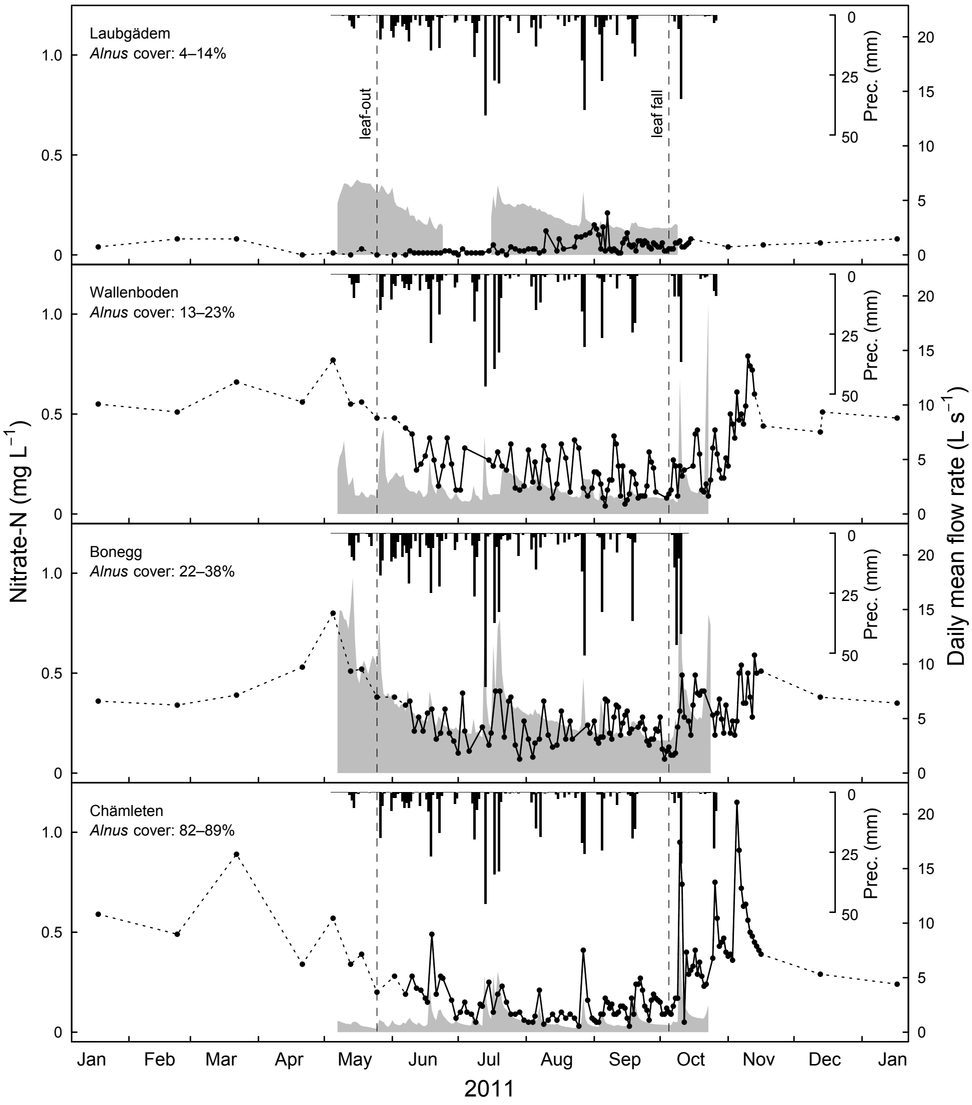

Figure 4.5: Daily mean flow rate (shaded), precipitation (black bars), and NO_3^--N concentration of the streamlets (dotted line: manual sampling) and precipitation. Note: flow rate and precipitation data were only available from May to October; missing flow data in Laubgädem (end June to mid July in 2011).

4. Discussion

By combining field measurements of N pools and fluxes, we provide clear evidence of the significant influence of *Alnus* on the ecosystem and catchment-wide N-balance. In contrast to the *Alnus* stands, no substantial N losses occurred in adjacent pastures despite regular grazing by cattle. Similar to North American *A. rubra* forests (Binkley et al., 1994), the soil solution under *A. viridis* stands in the Swiss Alps was highly enriched in NO_3^--N, and large NO_3^--N losses occurred, demonstrating that the biotic demand and the storage capacity for N of these soils were exceeded. Thus, according to the definition of Aber et al. (1989), the *Alnus* presence has driven these ecosystems into N saturation. The total N leaching during the growing season, as measured here under 50-year-old *Alnus* across all three valleys (0.69 to 0.89 g N m^{-2}) and 25-year-old *Alnus* in the Ursern Valley (1.76 g N m^{-2}), extrapolated to annual values (assuming the same leaching rate throughout the entire year as during the growing season) arrived at 1.7–4.2 g N m^{-2} yr^{-1} and thus, were similar to annual rates of 2.6 to 5.0 g N m^{-2} yr^{-1} under *A. rubra* forests reported by Binkley et al. (1992). The low leaching rates under Swiss montane coniferous forests (0–0.22 g N m^{-2} yr^{-1}) measured by Thimonier et al. (2010) and our own data for pastures demonstrate that current atmospheric N deposition did not yet cause substantial nitrate leaching in these montane ecosystems. Thus, atmospheric N deposition alone represents a minor N input relative to the N_2 fixation of *Alnus.*

The higher NO_3^--N concentration in the soil solution under 25-year-old compared to 60-year-old *Alnus* in the Ursern Valley, may be caused by higher N_2 fixation rates in younger stands as shown by Sharma et al. (2002). However, N_2 fixation rates were still substantial in mature North American and Himalayan *Alnus* stands (c. 6 g N m^{-2} yr^{-1}; Binkley et al., 1994). Here, we found similar amounts of NO_3^--N in the soil solution under 60-year-old and more than 100-year-old *Alnus* shrubland, therefore the *Alnus* induced N enrichment persisted for more than one century. Thus, a possible inhibition of N_2 fixation through increased soil N concentrations, commonly encountered under *Rhizobia* fixation (e.g., Streeter and Wong, 1988) was not detected. Earlier studies assumed that *Alnus* shrubland becomes fragmented after 40 to 70 years (Körner and Hilscher, 1978), however, such a decline was not observed here. Nitrate concentrations in the soil solution under *Alnus* at high elevation were 76% lower than those at low elevation. This elevation effect may be induced by decreasing N_2 fixation rates due to decreasing temperatures and/or shorter growing season at high elevation as emphasized by Sharma et al. (2010).

Suction cups have some limitations for mass balance calculations since they draw soil solution from variable space, depending on soil type, pore size and soil moisture (Weihermüller et al., 2007). Therefore, we had additionally employed zero-tension lysimeters. Yet, both methods yielded similar concentrations, irrespective of the amount of soil water they collected. Thus, we arrive at leaching rates that reflect the common soil solution concentrations of both collector types and the rainfall (seepage) regime and not the method employed. Since ammonium uptake by plant roots and nitrification release protons, and because nitrate leaching leads to losses of base cations (Blume et al., 2010), soil pH and actual base saturation were reduced under *Alnus* in comparison to pastures. Soil acidity under *A. viridis* has been shown to increase with the age of the shrubland (Caviezel et al., 2014). The observed changes in soil pH and base saturation over a few decades are indeed substantial, but line up with even more dramatic changes of 1.3 pH units observed in an Estonian *A. incana* afforestation over only 14 years (Uri et al., 2011).
The increased N concentration and the short-term N peaks after heavy rainfalls in streamlets in the catchments with an *Alnus* cover exceeding 20%, point at increasing N overflow responses (N saturation) as *Alnus* cover rises. All four test catchments were in topographically complex and steep terrain and were fed by N-poor (largely geological; Mueller et al., 2013) water, plus discharge from land surfaces above the treeline, thereby diluting any *Alnus* related signals. North American *A. rubra* (Compton et al., 2003), *A. viridis* and *A. incana* forests (Shaftel et al., 2012) led also to substantial NO_3^--N export into streams with a reported maximum concentration of 2.4 mg NO_3^--N L^{-1}. Similar short-term nitrate or DON pulses were found in North American *A. rubra* or mixed hardwood forests (Bechtold et al., 2003, Sebestyen et al. 2014). In streamlet water of micro-catchments with an *Alnus* cover >20% as well as in the soil solution under *Alnus*, the lowest NO_3^--N concentrations were commonly measured after the onset of the growing season, when plant N demand for biomass formation was highest. In autumn, NO_3^--N concentrations increased substantially in both, streamlet and soil solution due to reduced plant demand, large N inputs by the *Alnus* foliage and the fast decomposition of N-rich understorey plants. Thus, both NO_3^--N in soil solution and in streamlet water were closely coupled to each other, but were highly dependent on the type of plant cover, time of year and amount of precipitation.

The large N input by N_2 fixation did not only enlarge N fluxes but also represents an easily accessible N source for understorey plants. In general, δ^{15}N values of plant tissues mirror the isotopic signature of the N source and the fractionation during and after uptake (Högberg, 1997). Because discrimination during N_2 fixation is small, elevated δ^{15}N values in plants growing close to *Alnus* shrubs show that these plants incorporated N originally fixed by *Alnus* (Kohls et al., 2003). Also the higher leaf N concentrations of plants in the understorey of *Alnus* reflect the higher N availability. Intra- and interspecific variation in leaf δ^{15}N of non-fixing species was high, suggesting that these species have access to different N sources, whereas *Alnus* can be assumed to be self-sustaining in terms of N nutrition, since all tissues analysed in *Alnus* revealed high δ^{15}N values with very little intraspecific variation.

As we have shown, *Alnus* expansion leads to a large ecosystem N input, increased plant N pools, and a shift of the major phytomass N storage from belowground pools (pastures) to aboveground pools in *Alnus*, largely through the accumulation of wood mass. In contrast to our expectation, neither the total N nor the total C pools (phytomass plus soil) were increased in *Alnus* shrubland compared to pastures, and soil N and C pools tended to be lower under *Alnus* following from the significant lower N and C concentrations in the 2 mm soil fraction. Thus, the presence of *Alnus* appears to intensify the turnover of N and C in comparison to the pastures. Our findings contrast the results of Binkley et al. (1992) who found enlarged N and C soil pools in North American *A. rubra* forests, and similarly, increased atmospheric N deposition was found to augment C sequestration to soils (e.g., by 5–35 g C per g N in forests as reviewed by de Vries et al., 2009). Also *A. incana* stands on abandoned agricultural land in Estonia, showed N and C accumulation in soils (Uri et al., 2009). Neff et al. (2002) found accelerated decomposition of the light soil carbon fractions when N was added in dry meadows, while the heavier soil carbon compounds became further stabilized. Similar to our findings, soil C and N pools calculated from data by Caviezel et al. (2014), and by Hunziker and Caviezel (personal communication) also declined in 15-, 25- and 40-year-old *Alnus* stands compared to adjacent pastures in the Unteralp Valley, not far from our test site Oberalp. Paschke et al. (1989) explained reduced soil N pools in North American walnut interplantations with *A. glutinosa* and N_2-fixing *Elaeagnus umbellata* by higher N mineralisation, followed by higher N losses. Furthermore, partial and complete denitrification, causing gaseous N losses (NO, N_2O and N_2) most likely contributed to reduced soil N pools. Indeed, first results of our on-going research suggest large emissions of the greenhouse gas N_2O in the range of 0.1 to 0.4 g N_2O-N m^{-2} (from June to October) under *A. viridis* stands, while in pastures almost no N_2O was released (unpublished data). Thus, apart from nitrate leaching, denitrification

is another major pathway for N losses in *Alnus* stands. Gaseous N losses were also found to be higher in a German *A. glutinosa* forest (0.49 g N_2O-N m^{-2} yr^{-1}) compared to a beech forest (0.04 g N_2O-N m^{-2} yr^{-1}; Mogge et al., 1999). In an Estonian *A. incana* afforestation N was mainly emitted as N_2 (7.4 g N_2-N m^{-2} yr^{-1}) and to a lesser extent as nitrous oxide (0.05 g N_2O-N m^{-2} yr^{-1}; Uri et al., 2011). However, shifts between N_2O and N_2 (i.e., partial versus complete denitrification) are regarded as highly fluctuating in time and space.
Overall, we suggest that the high N leaching rates and gaseous N emissions explain why we did not find the expected increase in the total N pools (sum of phytomass and soil N pools) compared with adjacent pastures. In fact, there was a downward trend, which was not statistically significant, because soil bulk density varied so much among sites, so that the reductions in soil N and C concentrations in the 2 mm soil fraction did not significantly scale to pool size responses. We are confident that there were no a priori differences in soil N and C pools between pastures and *Alnus* before shrub encroachment took place, given the very close proximity, the identical aspect of the test sites and the similar results across three different valleys. N pools in pastures and *Alnus* were both calculated to a soil depth of 30 cm. However, the main rooting horizon in pasture was likely shallower than in the *Alnus* stands, so that the soil N pool might be higher in *Alnus*, when calculated for the entire rooting profile.

With the data available, we cannot close the N budget. Yet, if we assume a total annual N_2 fixation by *A. viridis* between 2.0–6.2 g N m^{-2} following Binkley (1981), add the modelled N deposition of 1–1.5 g N m^{-2} yr^{-1} for this region, and subtract the annual losses, namely leaching of 2.5 g N m^{-2} yr^{-1} (assuming similar rates during winter as during the growing season, supported by our intermittent winter data) and N_2O emissions of c. 1 g N m^{-2} yr^{-1} (assuming similar rates during winter as during the growing season), we arrive at an overall N balance between -0.5 and 4.2 g N m^{-2} yr^{-1} in the 50-year-old *Alnus* stands. However, taking into account the soil and phytomass N pools (and dividing by stand age), we arrived at a net change in total pools of -10.9 to -0.4 g N m^{-2} yr^{-1} at low and -4.3 to 2.8 g N m^{-2} yr^{-1} at high elevation, since *Alnus* has started to overgrow these former grasslands. Hence, the measured N fluxes cannot explain the changes of the total N pools in these *Alnus* stands. As already mentioned, large denitrification (NO, N_2O and N_2 emissions) may hold one explanation. Soil N pools may also have decreased more rapidly in the early stages of the shrub encroachment, given the 2–2.5-times higher N leaching under 25-year-old *Alnus* compared to 50-year-old stands.

While the land cover transition from pastures to *Alnus* shrubland did not yield higher N and C storage, *Alnus* also prevents potentially larger stocks by hindering forest succession (Bischof, 1984; Hiltbrunner et al., 2014). A montane forest at sites currently occupied by *Alnus* could stock 3 to 4.5-times more C (9,500–13,500 g C m^{-2}; FOEN and WSL, 2005). Therefore, *Alnus* shrubland is considered an undesirable land cover type from both a N and C balance perspective, in addition to its negative consequences on plant species richness (Bischof, 1984; Anthelme et al., 2007) and water relations (Bühlmann et al., 2014). We demonstrated that the ecosystem N enrichment by *Alnus* is substantial and far exceeds the critical loads for N in these montane ecosystems irrespective of elevation. Moreover, due to climate warming and reduced land use, the very likely expansion of the upper distribution limit of *Alnus* (Körner, 2012) will further lead to an encroachment into N sensitive, high-elevation ecosystems. Thus, the current rapid and massive expansion of *Alnus* in mountainous regions will persistently diminish plant diversity and drive these century-old grasslands into N saturated, species-poor, highly productive shrubland within a few decades.

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Supplementary material

Table S4.1: Dry weight (g m^{-2}), C concentration (mg C g^{-1} dry weight) and C pools (g m^{-2}) of plant material and soil (0–30 cm, mean ± se) of pastures and *Alnus* stands at low and high elevation.

	Pasture			*Alnus*		
	Dry weight	C conc.	C pool	Dry weight	C conc.	C pool
Low elevation						
Non-*Alnus* aboveground green phytomass						
Grasses	292 ± 103	454 ± 6	134 ± 49	9 ± 7	467 ± 23	4 ± 3
Herbs	73 ± 16	446 ± 5	33 ± 7	120 ± 57	440 ± 12	55 ± 26
Dwarf shrubs leaves[a]	3 ± 1	483 ± 9	1 ± 0	46 ± 42	503 ± 8	24 ± 22
Ferns	-	-	-	68 ± 13	462 ± 8	32 ± 7
Mosses	9 ± 2	401 ± 41	4 ± 1	11 ± 3	411 ± 22	5 ± 1
Total	377 ± 104	446 ± 42[#]	172 ± 50	255 ± 72	457 ± 35[#]	120 ± 35
Dwarf shrub stems[a]	6 ± 2	483 ± 9	3 ± 1	129 ± 117	504 ± 8	68 ± 62
Alnus aboveground phytomass[b]						
Leaves	-	-	-	311 ± 29	491 ± 6	152 ± 13
Phloem	-	-	-	858 ± 164	541 ± 4	464 ± 90
Xylem	-	-	-	4189 ± 801	480 ± 3	2001 ± 374
Total	-	-	-	5357 ± 818	-	2617 ± 385
Belowground phytomass						
Fine roots (< 2 mm)	1506 ± 279	401 ± 10	601 ± 100	251 ± 47	433 ± 16	111 ± 23
Roots (> 2 mm, not woody)	-	-	-	34 ± 14	451 ± 10	16 ± 6
Roots (> 2 mm, woody)	-	-	-	1216 ± 631	501 ± 4	612 ± 313
Rhizomes	-	-	-	102 ± 41	438 ± 15	43 ± 18
Nodules	-	-	-	13 ± 3	457 ± 10	6 ± 1
Total	1506 ± 279	401 ± 10	601 ± 100	1616 ± 634	456 ± 27[#]	789 ± 314
Total						
Phytomass	1889 ± 298	-	776 ± 112	7357 ± 1044	-	3593 ± 502
Litter[c]	39 ± 6	437 ± 15	17 ± 2	269 ± 21	527 ± 3	142 ± 11
Soil (0–30 cm)	-	79 ± 8	7912 ± 1786	-	51 ± 9	6186 ± 2212
Total (without litter)	-	-	8688 ± 1789	-	-	9779 ± 2268
High elevation						
Non-*Alnus* aboveground green phytomass						
Grasses	213 ± 56	461 ± 4	98 ± 27	6 ± 3	439 ± 7	3 ± 1
Herbs	77 ± 16	461 ± 5	36 ± 8	81 ± 37	446 ± 4	36 ± 17
Dwarf shrubs leaves[a]	26 ± 23	498 ± 8	13 ± 12	8 ± 6	500 ± 9	4 ± 3
Ferns	-	-	-	15 ± 15	479 ± 0	7 ± 7
Mosses	15 ± 11	380 ± 52	7 ± 6	1 ± 1	456 ± 2	1 ± 1
Total	331 ± 64	450 ± 53[#]	155 ± 31	111 ± 40	464 ± 12[#]	51 ± 19
Dwarf shrub stems[a]	24 ± 17	498 ± 8	12 ± 9	23 ± 18	500 ± 9	12 ± 9
Alnus aboveground phytomass[b]						
Leaves	-	-	-	291 ± 13	490 ± 3	142 ± 5
Phloem	-	-	-	655 ± 28	539 ± 5	351 ± 16
Xylem	-	-	-	3095 ± 175	486 ± 1	1500 ± 82
Total	-	-	-	4042 ± 178	-	1993 ± 84
Belowground phytomass						
Fine roots (< 2 mm)	1375 ± 366	450 ± 24	614 ± 146	271 ± 54	435 ± 6	117 ± 22
Roots (> 2 mm, not woody)	-	-	-	65 ± 21	447 ± 6	29 ± 10
Roots (> 2 mm, woody)	-	-	-	688 ± 336	498 ± 5	349 ± 178
Rhizomes	-	-	-	128 ± 37	448 ± 8	58 ± 18
Nodules	-	-	-	23 ± 10	460 ± 8	11 ± 4
Total	1375 ± 366	450 ± 24	614 ± 146	1175 ± 343	458 ± 15[#]	564 ± 181
Total						
Phytomass	1730 ± 372	-	781 ± 150	5351 ± 389	-	2619 ± 200
Litter[c]	34 ± 8	429 ± 7	14 ± 3	269 ± 16	523 ± 4	142 ± 8
Soil (0–30 cm)	-	63 ± 6	8090 ± 642	-	54 ± 5	6800 ± 629
Total (without litter)	-	-	8871 ± 659	-	-	9419 ± 660

[a] C concentration of dwarf shrubs was measured in bulk samples (leaves plus stems)

[b] Including *Sorbus* (on average below 6% of the total dry weight) and *Salix* (on average below 1% of the total dry weight)

[c] Canopy litter, including understorey/pasture litter collected manually in October

[#] Total concentration was calculated from means of 'aboveground green mass' or 'belowground mass'

Chapter 5

Nitrogen fixation by *Alnus* species boosts soil nitrous oxide emissions

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Abstract

The aim of this study was to assess how higher soil temperatures and increased nitrogen (N) availability, mediated by the N_2 fixation of different *Alnus* species, affect soil nitrous oxide (N_2O) emissions. We measured rates of N_2O emission *in situ* under non-N_2-fixing tree species or grassland (controls) and under *Alnus* species by the closed chamber technique along a temperature gradient of 5.7 K from the lowlands to the upper montane belt in Switzerland. Similar soil conditions and representative tree and bush taxa at a given elevation were a key prerequisite for the site selection. Across the twelve test plots, and irrespective of the elevation, N_2O emissions were on average 12-fold larger in *Alnus* stands than in non-N_2-fixing vegetation. The mean emission was 1.3 ± 0.2 kg N_2O-N ha^{-1} under *Alnus* and 0.1 ± 0.1 kg N_2O-N ha^{-1} under non-N_2-fixing vegetation from mid-June to mid-October. The largest rates of emission were observed in *Alnus viridis* (Chaix.) D.C. shrubland at 1680 m a.s.l. (mid elevation) with a seasonal emission of 4.2 ± 0.5 kg N_2O-N ha^{-1}. Differences in soil temperature along the elevation gradient did not affect the release of N_2O in non-N_2-fixing vegetation or across the different *Alnus* species. We conclude that larger N availability through N_2 fixation increases N_2O emissions in ecosystems even without intense agricultural management. The fast expansion of *Alnus viridis* across the Alps has been documented mainly in the context

of ‘ecological consequences’. Here, we provide evidence that *Alnus viridis* also adds substantially to greenhouse gas emissions.

1. Introduction

Human activities have altered the nitrogen (N) cycle considerably by increasing the release of reactive N by around 12-fold between 1860 and 2005, leading to larger N loads for all ecosystems (Galloway et al., 2008). In ecosystems with very little external inputs of N, the N cycle is largely closed and the demand for N by plants and microbes is mainly met by internal recycling (Schulze et al., 2002). Near this equilibrium (‘steady state’), N losses by N leaching and soil gaseous emissions are minor, and are offset by biological N_2 fixation or natural, low atmospheric N deposition. In ecosystems subjected to large external inputs of N from the application of N fertilizers, substantial atmospheric N deposition or biological N_2 fixation, the amount of N may exceed the biotic demand and buffering capacity of the soil. In such N saturated ecosystems, N will be lost through leaching, mainly in the form of nitrate, and by gaseous N emissions (Aber et al., 1989; Barnard et al., 2005). Accelerated nitrification and denitrification result in an increased release of nitrogen oxides (NO_x), nitrous oxide (N_2O) or molecular nitrogen (N_2) to the atmosphere.

Nitrous oxide is a strong greenhouse gas and an air pollutant. Its long atmospheric mean residence time of 114 years results in a warming effect per unit mass that is 265–310 times more than that of carbon dioxide (that corresponds to a global-warming potential (GWP) of 298, estimated for a 100-year period; Myhre et al., 2013). Moreover, N_2O also causes ozone depletion in the stratosphere. The majority of N_2O emissions originate from agriculture, mainly through the application of N fertilizers but also manure management. In Switzerland, 80% of the N_2O emissions are released from agriculture, and agriculturally used soil contributes c. 50% to the total emission within the agriculture sector (FOEN, 2015).

Little is known at present about the effect of increased N input (and thus greater N availability) and a warmer temperature on denitrification in unmanaged and close to natural ecosystems (Galloway et al., 2004; Barnard et al., 2005). Denitrification is co-determined by soil moisture and soil temperature, together with the amount and quality of available substrates (Blume et al., 2010). Some short-term laboratory studies have shown an increase in rates of denitrification with rising temperature (Stanford et al., 1975; Mogge et al., 1998), whereas others observed no effects of temperature on the release of N_2O (Smid and Beauchamp, 1976). Few field experiments only have assessed the effect of temperature on N_2O emissions under field conditions in close to natural ecosystems over longer periods (Carter et al., 2012; no effect was found). Atmospheric N deposition is well known to increase gaseous soil N emissions in close to natural ecosystems (Butterbach-Bahl et al., 2002; Kesik et al., 2005; Bühlmann et al., 2015). One of the largest annual rates of N_2O emission (5.1 kg N_2O-N ha^{-1} year^{-1}) was measured in an N saturated beech forest in southern Germany with a large rate of N deposition (20 kg N ha^{-1} year^{-1} measured in throughfall; Papen and Butterbach-Bahl, 1999). A few studies only have addressed the effect of N_2-fixing species on N_2O emissions in close to natural forests and shrubland (Mogge et al., 1998; Eickenscheidt et al., 2014).

For *Alnus* ssp.*,* N_2 fixation rates in the range of 20–150 kg N ha^{-1} year^{-1} have been reported (Binkley, 1981). Several studies have found larger amounts of N, mainly in the form of nitrate in the soil solution (Binkley et al., 1992; Bühlmann et al., 2016), and increased rates of denitrification under *Alnus* relative to non-N_2-fixing vegetation (Mogge et al., 1998). Nitrogen-fixing species may add to the N load from atmospheric N deposition by their N_2 fixation, especially in montane regions where rates of atmospheric N deposition are usually small (10–15 kg N ha^{-1} year^{-1}; Thimonier et al., 2005). Unlike other N_2-fixing species, *Alnus viridis* expands rapidly over wide areas in the montane zone (Hiltbrunner et al., 2014). Other *Alnus* species such as *Alnus incana* are naturally confined to riverine forests or lakeshores, and have been used in large-scale afforestation on former arable land (Uri et al., 2011). The increase in spatial extent of different *Alnus* species across the temperate and boreal zones emphasizes the importance of quantifying *in situ* the contribution of the genus *Alnus* to N_2O emissions.

The aim of this study was to explore the effect of temperature and increased N availability created by the presence of N_2-fixing trees and tall shrubs on soil N_2O emissions in close to natural ecosystems. Elevation or latitudinal gradients are considered 'natural experiments' that provide conditions under which denitrification and rates of N_2O emission reflect long-term adjustments to the prevailing temperatures. Here, we report N_2O fluxes from soil under different *Alnus* species and non-N_2-fixing species along a 1.65-km elevation gradient from the lowlands to the upper montane belt in Switzerland. We selected our study plots in either alluvial plains or moist, north- or east-facing slopes to cover plots that differed in vegetation and elevation, but had similar soil moisture conditions. At mid elevation (1200–1680 m a.s.l.), we included plots with two different *Alnus* species (*Alnus viridis* (Chaix.) D.C., *Alnus* *incana* L. Moench) and non-N_2-fixing plots with *Salix* sp. and pasture to analyse the effect of the land cover type on N_2O fluxes. We hypothesized that increased amounts of N from N_2 fixation prime N_2O emissions, and that this effect is more pronounced at the warmer lowland sites with a longer growing season.

2. Material and methods

2.1. Experimental design

Nitrous oxide emission, productivity and soil data were collected at sites along an elevation gradient from the Swiss Plateau to the Swiss Alps. Each site consisted of plots that differ in their natural N availability through the absence ('controls') or presence of N_2-fixing *Alnus* species (Table 5.1). At low elevation (290–330 m a.s.l.), two *Acer platanoides* L. forests were chosen as the controls and two *Alnus glutinosa* L. forests as plots with large N availability. At mid elevation (1190–1680 m a.s.l.), two out of three controls were dominated by *Salix fragilis* L. and the other was a pasture grazed by cattle. Two of the three *Alnus* plots were *Alnus incana* forests and one an *Alnus viridis* shrubland. At high elevation (1900–1950 m a.s.l.), a pasture was used as the control and an *Alnus viridis* stand was the plot with large N availability. We selected alluvial soils in river plains wherever possible (uniform moisture, well drained, similar bedrock chemistry). *Alnus viridis* and pasture plots were located on steep slopes, where soil chemistry, moisture and drainage were similar to the alluvial soils. All plots had a homogenous plant cover extending over an area of at least 60 m × 60 m, and the sampled zone was surrounded by more than 10 m of the same vegetation type.

Soil temperature was recorded hourly at a depth of 10 cm at each plot (Tidbit v2 Temp logger, Onset Computer Corp., Bourne, MA, USA). These temperature records were used to define the length of the thermal growing season by the number of days with a daily mean soil temperature $>$ 5 °C. Data on precipitation were obtained from MeteoSwiss and Bühlmann et al. (2016) and modelled atmospheric nitrogen deposition was provided by FOEN/Meteotest Switzerland. Peak season leaf area index (LAI) was estimated by measuring light transmission and by applying the light extinction law (ceptometer, AccuPAR LP-80, Decagon Devices Inc., Pullman, WA, USA). In *Alnus viridis* plots, LAI was also assessed by allometric relations (Bühlmann et al., 2016). The stand age was estimated from the age of the thickest stems. Soil pH (0–10-cm depth) was measured on one pooled sample per plot in 0.1 M KCl in *Acer, Salix, Alnus glutinosa* and *Alnus incana* stands and in five soil samples per plot in 0.01 M $CaCl_2$ in pastures and *Alnus viridis* shrubland (different methods resulted here from different field surveys). Soil bulk density and the N and C concentrations of the 2-mm soil fraction (measured by an elemental analyser, FlashEA 1112, ThermoFinnigan, Milan, Italy) were analysed in samples taken at 0–30-cm soil depth.

2.2. Soil N_2O emissions

Soil–atmosphere N_2O fluxes were measured *in situ* by a closed chamber technique every two weeks (Hartmann and Niklaus, 2012). Soil N_2O emissions in *Acer, Salix, Alnus glutinosa* and *Alnus incana* forests were measured between mid-June and mid-October in 2009, and in pastures and *Alnus viridis* during the same months but three and four years later (2012, 2013). Within each plot, six chamber collars with detachable lids (20 cm in height, 32 cm in diameter) were installed at random locations (zones with thick roots were avoided) two weeks prior to the first measurement. After pre-trenching the soil with a shovel, the chamber collars were inserted 10 cm into the ground, resulting in an average headspace volume of 8.5 l per chamber. To follow the common grazing regime in this region, the plant canopies in pastures were cut to a stubble height of 4 cm inside the chambers at the beginning of August in both years. The chambers were closed at the time of measurement and headspace samples were taken after 5, 20 and 35 minutes and injected into pre-evacuated exetainers. Nitrous oxide concentrations of the samples were measured with a gas chromatograph (Agilent 7890 equipped with an electron capture detector, Agilent Technologies Inc., Santa Clara, CA, USA). Nitrous oxide flux rates were calculated by linear regression of the N_2O concentrations of the three air samples taken from the headspace against sampling time (R^2 was generally above 0.98), and expressed in μmol N_2O m^{-2} day^{-1} using the ideal gas law. Seasonal or annual rates of emission were estimated by multiplying the mean across all daily rates of emission by the number of days of the period of interest.

In addition to the continuous measurements of soil temperature, it was recorded manually and concomitantly with the N_2O flux measurements at a soil depth of 10 cm next to each chamber (digital thermometer GTH 175/Pt, Greisinger Electronic, Regenstauf, Germany). We took soil moisture readings manually (ThetaProbe - ML2x, Delta-T Devices Ltd., Cambridge, UK) in 2009 and continuously in 2012 and 2013 (10HS soil moisture probes installed at a depth of 10 cm; Decagon Devices Inc., Pullman, WA, USA).

2.3. Potential nitrification (NEA) and denitrification enzyme activity (DEA)

Soil samples for nitrification enzyme activity (NEA) and denitrification enzyme activity (DEA) measurements were collected with a soil corer (depth 10 cm, 2.5 cm in diameter) at 10 randomly distributed locations within each plot. The samples were pooled for each plot, sieved (2-mm mesh) and kept at 4 °C until enzymatic assays (2–7 days) were made. Soil water content was determined gravimetrically (10-g sieved soil, 105 °C, 24 hours).

The NEA was estimated as NO_3^- production in the presence of excess NH_4^+ in a short-term laboratory incubation in soil samples from *Acer*, *Salix*, *Alnus glutinosa* and *Alnus incana* plots in June 2009 (Schmidt and Belser, 1994). Fresh, sieved soil, equivalent to 10 g dry mass, was mixed with 100 ml of buffer solution (0.5 mM KH_2PO_4/K_2HPO_4, adjusted by adding K_2CO_3 to achieve a pH of 7; optimal conditions for nitrifiers) and incubated at 25 °C. We added 1 ml 0.05 M $(NH_4)_2SO_4$ to the soil suspension. Aliquots were taken after 1, 4, 7 and 23 hours, centrifuged, filtered and analysed for NO_3^- concentrations by flow-injection analysis (Skalar SAN++, Skalar, Breda, The Netherlands). Potential rates of nitrification were calculated by linear regression of NO_3^- concentrations against sampling time.

The DEA was measured twice for all plots as a short-term potential of N_2O production under optimized conditions for enzymatic activity (excess N and organic C, no oxygen and no diffusion limitation; Tiedje et al., 1989) in June and in August or September. Fresh soil, equivalent to 5 g of dry mass, was weighed into a 150 ml serum bottle and 4 ml demineralized water were added. To create oxygen-free conditions, the headspace was evacuated twice and replaced by 9:1 $He:C_2H_2$. We added 1 ml of a solution containing 1 mg glucose-C g^{-1} dry soil, 1 mg glutamic acid-C g^{-1} dry soil and 0.1 mg NO_3^--N g^{-1} dry soil as C and N substrates. Incubation at 25 °C was kept short to minimize *de novo* synthesis of denitrifying enzymes: after 40, 80 and 120 minutes headspace samples were taken and analysed for N_2O concentration by gas chromatography. The DEA was calculated by linear regression of N_2O concentrations against time.

2.4. Aboveground productivity

As an indicator for aboveground productivity, we collected canopy and understorey litter (woody parts excluded) in forests and shrubland and aboveground phytomass in pastures. For forests and shrubland, we refer to the sum of the canopy litter and understorey peak-season phytomass as the total litter production. We collected canopy litter every two weeks with randomly placed traps (area: 0.25 m^2): there were six traps per plot in *Acer, Salix, Alnus glutinosa* and *Alnus incana* stands in 2009 and three traps (area: 0.2 m^2) per plot under *Alnus viridis* in 2012. Understorey phytomass was harvested in one 1 m × 1 m quadrat in *Acer, Salix, Alnus glutinosa* and *Alnus incana* plots, and in five 0.5 m × 0.5 m quadrats in *Alnus viridis* plots because its understorey is more patchy. In pasture plots, dry weight of peak-seasonally harvested phytomass of five 0.25 m × 0.25 m quadrats were used to estimate annual litter production. All samples were dried at 80 °C and weighed.

2.5. Statistical analyses

All statistical analyses were carried out using R (R 3.2, R Development Core Team, 2013). Effects of the presence of N_2-fixing vegetation (a two-level factor: control and N_2-fixing) and elevation (a continuous variable with values of 1, 2 and 3 for low, mid and high elevations, respectively) on the dependent variables N_2O emission, NEA, DEA and litter production were tested by the analysis of variance based on linear models. Reflecting the design of the experiment with plots nested within site, the models were hierarchical, with 'site' specified as the error term. Effects of elevation were tested using site as replicate, whereas effects related to the presence or absence of N_2-fixing vegetation were tested using plot as a replicate. As measure of N_2O emissions, we used growing season means of N_2O fluxes from individual chambers in each plot. These data were log-transformed (natural logarithm), which yielded normally distributed and homoscedastic residuals (confirmed by inspection of normal quantile plots). Specific hypotheses (e.g. the effects of *Alnus* species within a given elevation level) were tested using linear contrasts within the overall linear model. All data are reported as means and standard error of the raw data (mean ± SE). We report the significance of differences between means based on the ANOVA.

3. Results

3.1. Environmental conditions

Mean annual soil temperatures ranged from 10.4 °C at low to 4.7 °C at high elevations, resulting in a temperature gradient of 5.7 K along the 1.65-km elevation gradient (Table 5.1). The soil temperature gradient was less steep than that commonly expected from the adiabatic lapse rate of air temperature (Körner, 2003; 0.6 K per 100-m elevation would amount to 9.9 K along the 1.65-km gradient). Except for the pastures, soil temperatures were monitored under dense tree and shrub canopies, which explains the less steep temperature gradient overall. There were minor differences in temperature only between the control and N_2-fixing plots at the same elevation. Mean soil temperature during the growing season was 14.8 °C at low, 10.5 °C at mid and 10.7 °C at high elevation. The growing season lasted on average 180 days at low, 150 days at mid and 125 days at high elevations. At low elevation, the forest floor was only periodically covered with a few centimetres of snow, whereas at mid and high elevations a large snow pack of 1–2 m lasted from the beginning of December to mid-May. Precipitation from June to October increased from 330 mm at low to 560 mm at high elevations. Soil moisture in the topsoil varied only little throughout these months and among plots, and the soil at all plots was moist but well drained during the measurement periods. Soil pH was 6.1 at low elevation and 4.6 for both mid and high elevations (Table 5.2). Differences in pH among vegetation types were small, except for the high elevation sites where the soil pH under *Alnus* was one pH unit less than in the adjacent pastures. Soil bulk density varied considerably between plots. Soil C:N ratio was 11.1 at low, 16.1 at mid and 11.4 at high elevations. The larger C:N ratio at mid elevation resulted from one extreme value of 36.8 in an *A. incana* stand.

Table 5.1: Location of the study sites, stand age, LAI (mean ± SE), litter (sum of canopy and understorey litter, peak season phytomass in pastures), soil temperature at 10-cm soil depth (absolute minimum, maximum in parentheses), length of growing season, precipitation and modelled atmospheric nitrogen deposition for 2010. For pastures and *Alnus viridis* data of litter, temperature and soil moisture of 2012 are shown.

Elevation and vegetation m a.s.l.	Location	Species	Stand age (years)	LAI (m^2 m^{-2})	Litter (g m^{-2})	T_{soil} full year (°C)	T_{soil} growing season (°C)	Growing season[1] (days)	Precipitation June–Oct (mm)	N deposition[2] (kg N ha^{-1} $year^{-1}$)
Low										
Non-N_2-fixers										
296	47°32'37'' N / 7°46'22'' E	*Acer platanoides*	50–60	4.7 ± 0.1	723	10.1 (0.6, 21.8)	14.8 (6.4, 20.6)	179	330[3]	32.0
330	47°32'53'' N / 8°13'34'' E	*Acer platanoides*	40–50	5.0 ± 0.2	682	10.3 (1.6, 19.6)	14.8 (6.5, 19.2)	179	370[3]	19.7
N_2-fixers										
300	47°32'42'' N / 7°46'07'' E	*Alnus glutinosa*	110–150	5.5 ± 0.1	619	10.4 (0.9, 20.5)	14.8 (6.7, 19.7)	179	330[3]	32.0
328	47°32'49'' N / 8°13'38'' E	*Alnus glutinosa*	c. 100	5.9 ± 0.2	737	10.3 (1.6, 19.6)	14.8 (6.5, 19.2)	179	370[3]	19.7
Mid										
Non-N_2-fixers										
1515	46°36'16'' N / 8°31'01'' E	*Salix fragilis*	c. 40	3.7 ± 0.2	461	5.9 (0.5, 16.4)	11.0 (5.3, 15.1)	155	470[3]	12.7
1508	46°36'21'' N / 8°31'21'' E	*Salix fragilis*	c. 40	4.1 ± 0.1	475	5.5 (0.1, 14.7)	10.4 (5.2, 13.7)	153	470[3]	12.7
1675	46°36'49'' N / 8°34'01'' E	Pasture	-	-	112	5.2 (0.3, 14.2)	10.5 (5.4, 13.6)	139	480[4]	10.0
N_2-fixers										
1390	46°32'13'' N / 8°21'30'' E	*Alnus incana*	40–50	4.9 ± 0.2	372	5.7 (0.4, 14.9)	10.1 (5.4, 13.4)	159	310[3]	13.7
1190	46°43'02'' N / 8°54'09'' E	*Alnus incana*	40–50	4.4 ± 0.1	399	7.5 (0.6, 16.3)	12.0 (5.1, 15.7)	168	420[3]	14.9
1684	46°36'48'' N / 8°34'02'' E	*Alnus viridis*	c. 60	3.4 ± 0.3	243	4.3 (0.6, 12.3)	9.2 (5.2, 11.8)	126	480[4]	11.9
High										
Non-N_2-fixers										
1955	46°35'16'' N / 8°25'54'' E	Pasture	-	-	64	4.7 (0.4, 14.8)	10.8 (5.1, 13.6)	124	560[4]	8.9
N_2-fixers										
1901	46°35'31'' N / 8°29'08'' E	*Alnus viridis*	c. 50	3.7 ± 1.6	261	4.9 (0.8, 15.8)	10.5 (6.3, 14.6)	125	560[4]	10.4

[1] days with daily mean temperature above 5 °C

[2] data provided by FOEN/Meteotest Switzerland

[3] data provided by MeteoSwiss

[4] Bühlmann et al. (2016)

Table 5.2: Soil pH (0–10-cm soil depth), soil bulk density (0–30 cm), soil C:N ratio (0–30 cm) and soil moisture (mean ± SE). Note: For *Acer platanoides*, *Alnus glutinosa*, *Salix fragilis* and *Alnus incana* the mean of both sites is listed, and for pastures and *A. viridis* data of 2012 are given.

Elevation	Species	Soil pH	Soil bulk density (g cm^{-3})	Soil C:N ratio	Soil moisture June–Oct (% vol)
Low					
Non-N_2-fixers					
	Acer platanoides	6.3 ± 0.4	1.14 ± 0.06	11.1 ± 1.2	33.5 ± 0.4
N_2-fixers					
	Alnus glutinosa	6.0 ± 0.7	0.78 ± 0.10	11.2 ± 1.9	48.9 ± 1.3
Mid					
Non-N_2-fixers					
	Salix fragilis	5.3 ± 0.1	1.11 ± 0.9	12.4 ± 6.1	26.8 ± 0.8
	Pasture	4.0 ± 0.1	0.55 ± 0.03	13.2 ± 0.8	37.7 ± 1.1
N_2-fixers					
	Alnus incana	5.5 ± 0.7	1.04 ± 0.09	23.8 ± 6.9	30.0 ± 1.9
	Alnus viridis	3.6 ± 0.1	0.55 ± 0.03	15.3 ± 1.9	37.7 ± 1.1
High					
Non-N_2-fixers					
	Pasture	5.1 ± 0.1	0.54 ± 0.03	12.5 ± 0.4	33.6 ± 1.1
N_2-fixers					
	Alnus viridis	4.1 ± 0.1	0.51 ± 0.02	10.2 ± 0.5	28.7 ± 1.7

Modelled atmospheric N deposition was largest at low elevation (19.7–32.0 kg N ha^{-1} year^{-1}) and decreased to 8.9–10.4 kg N ha^{-1} year^{-1} at high elevation. All forest or shrubland stands were 40–150 years old; the *Alnus glutinosa* stands at low elevation had the greatest age. The canopy LAI of 5.3 ± 0.1 at low elevation was smaller than that at mid elevation (4.1 ± 0.1) and tended to decrease to 3.7 ± 1.6 at high elevation. The LAI was 20% larger in *Alnus* stands than in the non-N_2-fixing forests (pastures and *A. viridis* were excluded here because of different growth forms). The total amount of canopy and understorey litter did not differ among non-N_2-fixing and *Alnus* plots, except at high elevation where litter production was four times less in pastures than in shrubland, mainly because of the different growth forms. Overall, the total amount of litter, decreased significantly with elevation (691 ± 26 g m^{-2} at low, 344 ± 57 g m^{-2} at mid, 163 ± 99 g m^{-2} at high elevation; $F_{1,4} = 16.9$, $P < 0.05$).

3.2 Nitrous oxide emission

During the period from June to October, all chambers had mean net rates of N_2O release that ranged from close to zero to 90 μmol N_2O m^{-2} day^{-1}. One chamber located in the mid elevation pasture, however, showed a negligible mean net N_2O uptake of –2.0 to –0.8 μmol N_2O m^{-2} day^{-1} in 2012 and 2013. We did not detect a seasonal pattern for N_2O emissions under either non-N_2-fixers or in *Alnus* stands (Figure 5.1 and Figure S5.1, Supporting Information). Large peaks of N_2O emissions occurred in *Alnus* stands only and the large releases depended mainly on the rates of emission of one or two chambers per plot. These N_2O peaks could not be explained by either changes in soil moisture or changes in soil temperature. Individual chambers in *Alnus* stands repeatedly had rates of N_2O emission above 100 μmol N_2O m^{-2} day^{-1} (the single largest rate was 504 μmol N_2O m^{-2} day^{-1} measured under *Alnus viridis* at mid elevation). Under non-N_2-fixing vegetation, rates of N_2O emission were below 10 μmol N_2O m^{-2} day^{-1} for 95% of all measurements (maximum rate of emission was 67 μmol N_2O m^{-2} day^{-1} measured in an *Acer platanoides* forest at low elevation). Overall, the mean rates of N_2O emission were 12-fold larger in *Alnus* stands than for all non-N_2-fixing types of vegetation and plots during the growing season (Figure 5.2; $F_{1,4} = 8.8$, $P < 0.05$; Table S5.1, Supporting Information). This large difference in N_2O emissions between N_2-fixing and non-N_2-fixing vegetation was caused mainly by *A. viridis* shrubland and one *A. glutinosa* plot. Under non-N_2-fixing vegetation and *Alnus*, there was no correlation between N_2O emissions and elevation ($F_{1,4} = 0.1$, $P = 0.98$). At plots where N_2O emissions were measured throughout two growing seasons, rates of emission did not differ between seasons (2012, 2013; Figure S5.1, Supporting Information). At mid elevation, the mean N_2O emission was similarly small under both non-N_2-fixing vegetation types (*Salix*: 1.3 ± 0.5 μmol N_2O m^{-2} day^{-1}; pasture: 2.0 ± 1.1 μmol N_2O m^{-2} day^{-1}), but differed markedly among the *Alnus* plots. The mean N_2O emission was much larger under *Alnus viridis* (123 ± 18 μmol N_2O m^{-2} day^{-1}) than for *Alnus incana* stands (4.6 ± 0.6 μmol N_2O m^{-2} day^{-1}; $F_{1,5} = 13.5$, $P < 0.05$).

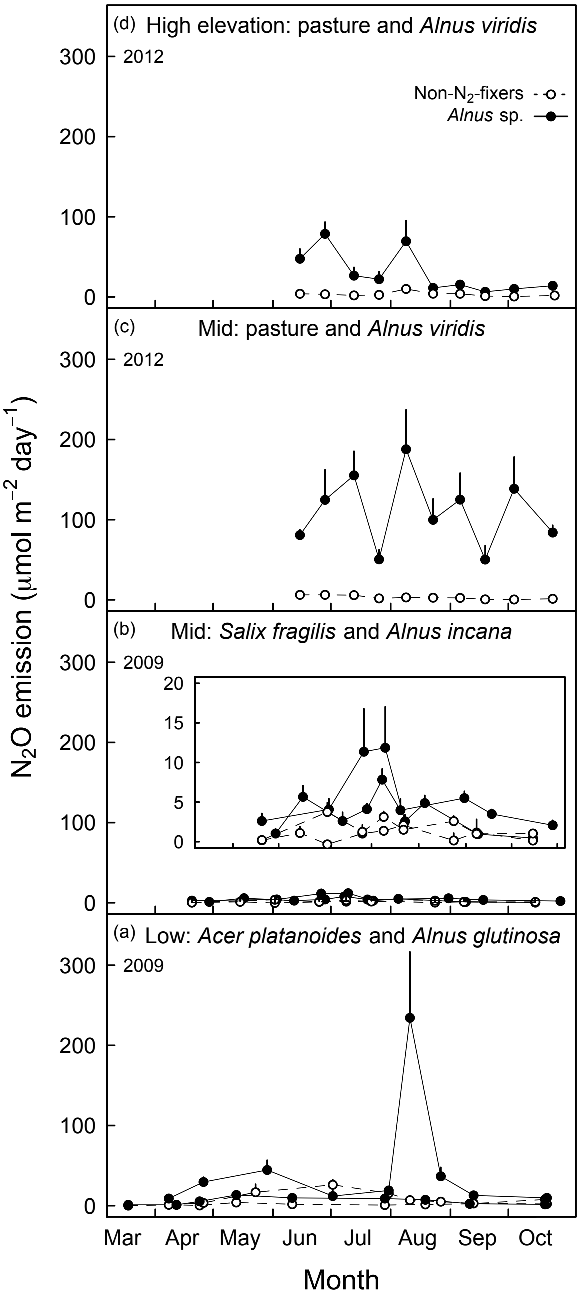

Figure 5.1: Mean rates of daily soil N_2O emission under non-N_2-fixers and different *Alnus* species at low (a), mid (b, c) and high (d) elevations (mean + 1 SE of the raw data; note: rates of N_2O emission of pastures and *Alnus viridis* in 2013 are shown in Figure S5.1; enlarged figure within the figure of *Salix fragilis* and *Alnus incana*; there were two *Salix fragilis* and *Alnus incana* plots at mid elevation and two *Acer platanoides* and *Alnus glutinosa* plots at low elevation).

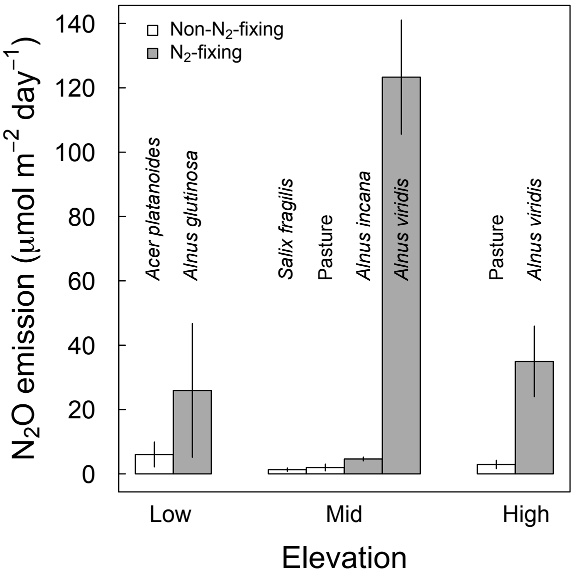

Figure 5.2: Mean N_2O emission under non-N_2-fixers and *Alnus* species at three elevations during the growing season (mean ± SE of the raw data; note: for pastures and *Alnus viridis* 2012-data are shown).

Under non-N_2-fixers, the measured rates of emission scaled to a seasonal sum was 0.1 ± 0.1 kg N_2O-N ha^{-1} from mid-June to mid-October (Table 5.3). During the same period, soil across all *Alnus* stands released on average 1.3 ± 0.2 kg N_2O-N ha^{-1}. Surprisingly, the largest rate of emission occurred under *Alnus viridis* shrubland at mid elevation (4.2 ± 0.5 kg N_2O-N ha^{-1} $season^{-1}$) and even at high elevation (1901 m a.s.l.) the emission under this *Alnus* species was still substantial (1.2 ± 0.3 kg N_2O-N ha^{-1} $season^{-1}$).

3.3 Potential nitrification and denitrifying enzyme activity

In addition to larger rates of N_2O emission, potential nitrification enzyme activity (NEA) was also larger in soil from *Alnus* stands than in soil from non-N_2-fixing vegetation ($F_{1,8} = 24.3$, $P < 0.05$) and tended to be larger at low than at mid elevations ($F_{1,8} = 8.8$, $P < 0.1$; NEA for pastures and *Alnus viridis* are not available).

Similar to NEA, denitrifying enzyme activity (DEA) was greater in soil under *Alnus glutinosa* than under *Acer platanoides*, and also in soil under *Alnus incana* than that under *Salix fragilis* (Table 5.3). Unexpectedly, DEA measured in pastures was larger than that under *Alnus viridis* at mid and high elevations. Thus, the effect of the presence of *Alnus* on the DEA became statistically non-significant. Nevertheless, DEA was still larger in *Alnus viridis* than under all other *Alnus* stands, indicating a considerable potential for denitrification in soil under this *Alnus* species.

Table 5.3: Potential nitrification (NEA), denitrification enzyme activity (DEA) and estimated total N_2O-N emissions from mid-June to mid-October (mean ± SE).

Elevation	Species	NEA (μmol NO_3^--N (g soil)$^{-1}$ day^{-1})	DEA (nmol N_2O (g soil)$^{-1}$ hour^{-1})	N_2O emission June–Oct (kg N_2O-N ha^{-1})
Low				
Non-N_2-fixers				
	Acer platanoides	157.3 ± 24.5	6.15 ± 0.4	0.10 ± 0.04
	Acer platanoides	43.4 ± 0.7	9.4 ± 4.9	0.33 ± 0.10
N_2-fixers				
	Alnus glutinosa	285.6 ± 33.0	18.8 ± 8.7	0.20 ± 0.05
	Alnus glutinosa	128.8 ± 22.4	15.1 ± 0.3	1.65 ± 0.74
Mid				
Non-N_2-fixers				
	Salix fragilis	4.0 ± 0.4	0.3 ± 0.1	0.03 ± 0.02
	Salix fragilis	4.0 ± 0.7	0.5 ± 0.1	0.06 ± 0.02
	Pasture	-	71.1 ± 5.5	0.07 ± 0.021
N_2-fixers				
	Alnus incana	144.7 ± 19.2	5.5 ± 0.5	0.14 ± 0.02
	Alnus incana	38.4 ± 3.5	1.8 ± 0.2	0.18 ± 0.08
	Alnus viridis	-	45.6 ± 4.4	4.21 ± 0.461
High				
Non-N_2-fixers				
	Pasture	-	64.7 ± 4.0	0.10 ± 0.031
N_2-fixers				
	Alnus viridis	-	32.3 ± 2.8	1.19 ± 0.341

4. Discussion

The large N input by *Alnus'* N_2 fixation clearly caused substantial N_2O emissions, whereas the N_2O losses were generally very small under non-N_2-fixing vegetation. Because soil conditions and atmospheric N deposition at plots with the same elevation were similar, N_2 fixation by *Alnus* is the most likely source of these N inputs. Widespread montane plant species such *Solidago virgaurea*, *Rhododendron ferrugineum* and *Vaccinicum myrtillus* hand strongly enriched foliar δ^{15}N values when growing beneath *A. viridis* canopies only, demonstrating that these species readily took up and incorporated nitrogen originating from fixation without $^{14}N/^{15}N$ isotope fractionation (Bühlmann et al., 2016). The variation in soil temperature along our elevation gradient of 1.65 km could not explain the rates of N_2O emission.

Rates of N_2O emission of non-N_2-fixing deciduous forests with little atmospheric N deposition were reported to be in the range of 0.1–0.5 kg N_2O-N ha^{-1} year^{-1} (Mogge et al., 1998; Kesik et al., 2005), which was similar to what we found in non-N_2-fixing tree stands. Papen and Butterbach-Bahl (1999), however, reported annual rates of N_2O release of up to 5.1 kg N_2O-N ha^{-1} year^{-1} in forest areas heavily polluted with N in southern Germany. The seasonal N_2O emissions in our montane pastures (0.1 kg N_2O-N ha^{-1}) were slightly smaller than the rates measured recently in Swiss montane pastures at 1975 m a.s.l. (c. 0.3 kg N_2O-N ha^{-1}, extrapolated from data of Hartmann and Niklaus, 2012) and in the Austrian Alps at 970 m a.s.l. (0.68 kg N_2O-N ha^{-1} year^{-1}; Hörtnagl and Wohlfahrt, 2014). Thus, rates of N_2O emission in close to natural ecosystems such as forests and unfertilized grasslands are commonly less than 1 kg N_2O-N ha^{-1} year^{-1}. These rates contrast considerably with those of N_2O emission in grasslands under intense agricultural management with ploughing and fertilizer application (111 kg N ha^{-1} year^{-1}), where emissions of 29.1 kg N_2O-N ha^{-1} year^{-1} with daily peak rates of emission of up to 6000 μmol N_2O m^{-2} day^{-1} have been measured by the eddy covariance technique in the Swiss plateau (Merbold et al., 2014).

Our rates of emission for *Alnus glutinosa* forests in the lowlands (0.2–1.7 kg N_2O-N ha^{-1}, June to October), extrapolated to one year, were similar to the annual rates of *Alnus glutinosa* forests (0.5–1.0 kg N_20-N ha^{-1} year^{-1}) in southern Germany (Eickenscheidt et al., 2014) and in northern Germany (0.8–7.3 kg N_2O-N ha^{-1} year^{-1}, Mogge et al., 1998). Furthermore, the emission rates of 0.1–0.2 kg N_2O-N ha^{-1} season^{-1} that we recorded in *Alnus incana* forests at mid elevation were in accord with those reported for *Alnus incana* forests in Estonia under short-term rotation management (0.5 kg N_2O-N ha^{-1} year^{-1}; Uri et al., 2011).

The largest rate of N_2O emission by far of all our *Alnus* plots occurred under *Alnus viridis* at mid elevation with a rate of 4.2 kg N_2O-N ha^{-1} season^{-1} and at high elevation with a rate of 1.2 kg N_2O-N ha^{-1} season^{-1}. Schürmann et al. (2002) reported a smaller emission of 0.4 kg N_2O-N ha^{-1} from soil under *Alnus viridis* in southern Switzerland at a similar elevation (1960 m a.s.l.) during the snow-free period. Interestingly, they found slightly larger N_2O emissions throughout the winter period in the same study (0.5–0.8 kg N_2O ha^{-1}). In winter, N_2O emissions can contribute substantially to the total annual N_2O budget because parts of the microbial biomass collapses during frost periods, and easily degradable N and C compounds become available for denitrifying microbes without competition from plant roots at this time of the year. For example, in a beech and spruce forest in South Germany, 39% and 73% of the annual total N_2O emission were released during long-term frost periods and subsequent thawing events (Papen and Butterbach-Bahl, 1999). At our mid and high plots with *Alnus viridis* and *Alnus incana*, however, permanent, deep snow packs during winter protected the soil from recurrent freezing and thawing periods (soil temperature never dropped below zero; Table 5.1).

Overall, the small N_2O emissions from soil under non-N_2-fixing vegetation corroborate the view that denitrification in the forest and grassland types studied is most probably limited by the availability of N, which was indicated by Carter et al. (2012) in peatlands and shrublands across Europe. In contrast, the large rates of N_2O emission from soil under *Alnus*, especially under *A. viridis*, suggest that inputs of N by N_2 fixation lead to an excess of reactive N with the result that the soil and ecosystems are saturated with N. Consequently, N losses occur mainly through nitrate leaching and, as seen in this study, through substantial gaseous losses (Binkley et al., 1992; Uri et al., 2011; Bühlmann et al., 2016). Our recent analysis of the N fluxes and pools in *Alnus viridis* shrubland revealed that despite the large N inputs through N_2 fixation, N did not accumulate in the soil, which means that there was no increase in soil N stocks because of these continuously large N losses (Bühlmann et al., 2016). In addition, soil C pools also decreased and levelled out with the larger phytomass C pools at the ecosystem scale (Bühlmann et al., 2016).

The increased availability of N explains the larger NEA and DEA in the presence of N_2-fixing species such as *Alnus* or legumes (Le Roux et al., 2013). The DEA was only less in *Alnus viridis* than in the adjacent pastures. Nevertheless, DEA was still twice as large in *Alnus viridis* than in *Alnus glutinosa* stands (largest DEA measured across all non-*Alnus viridis* plots). The NEA assays for soil samples taken from *Alnus viridis* stands and pastures did not provide meaningful results, probably because of unknown soil matrix effects. In acidic soil, heterotrophic nitrifying bacteria, Archaea and fungi commonly contribute to the nitrification (De Boer and Kowalchuk, 2001; Leininger et al., 2006), and especially nitrifying activities by fungi, which have not been included here. We do not know whether this was the case at the plots with acidic soil and to what extent this affected our enzyme assay under laboratory conditions.

Although some studies showed that higher temperatures and greater soil moisture increase N_2O emissions in the short term, this was for the duration of days and months (Mogge et al., 1998; Eickenscheidt et al., 2014), for others, including the present study, this was not the case (Pfenning and McMahon, 1997; Hartmann and Niklaus, 2012). In the short term, either temperature and soil moisture did not affect N_2O emissions because of limitation by other factors such as low C or N availability, or short-term peak emissions have been missed because sampling frequency was too small. Continuous measurements by eddy flux towers and automated chamber systems equipped with a quantum cascade laser have shown that such peaks arise within less than one hour and are often missed by the closed chamber technique (Merbold et al., 2014; Savage et al., 2014). In our *Alnus* stands, the species identity or plot effects, or a combination of the two, had a stronger effect on the release of N_2O than a possible long-term temperature effect (elevation and temperature gradient, respectively).

Testing the species effect across the whole elevation gradient was not possible because not all *Alnus* species occurred at all elevations due to their different distribution ranges. At mid elevation, however, we have data for two *Alnus* species. Here, the *Alnus incana* forests clearly released less N_2O than *Alnus viridis* shrubland. The substrate might have contributed to this difference because *Alnus incana* was growing on alluvial plains, whereas *Alnus viridis* was spreading on former pasture slopes. There are no differences in emissions of N_2O, however, among the corresponding non-N_2-fixing vegetation types (*Salix* stand and pasture) at mid elevation, suggesting a strong effect of the different *Alnus* species.

Aboveground productivity did not exert an effect on the release of N_2O because productivity decreased from 691 ± 26 g m^{-2} at our low elevation site to 163 ± 99 g m^{-2} at the high elevation sites but N_2O emissions did not. Litter production did not differ significantly between non-N_2-fixing vegetation and *Alnus* stands. Therefore, N_2 fixation in *Alnus* stands is the major factor in controlling increased emissions of N_2O across our plots.

Alnus viridis is currently encroaching large boreal areas in Alaska, Northwest Canada, Northern Europe, Siberia and the European Alps. In central Europe, *A. viridis* is the shrub species that is expanding most in terms of land cover and is rapidly encroaching former, century-old montane grassland in the Austrian, French, Italian and Swiss Alps (Bühlmann et al., 2014). It forms dense stands that persist for centuries and prevent succession towards montane forests (Hiltbrunner et al., 2014 and citations therein). In Switzerland, in total 48 500 ha of montane terrain have already been overgrown by *Alnus viridis* (data provided by www.lfi.ch, for the years 2004–2006) and the shrub is presently expanding at an annual rate of c. 1000 ha $year^{-1}$ (calculated from data of Brändli, 2010). In contrast to *A. viridis,* the spatial extent of *A. incana* and *A. glutinosa* is small in Switzerland.

Because the rates of N_2O emission of *Alnus viridis* are substantial and its areal extent is also large, we estimated the total seasonal N_2O emission of *Alnus viridis* stands in Switzerland. The total area of *Alnus viridis* shrubland below 1800 m a.s.l. (data provided for the years 2004–2006 by www.lfi.ch) was multiplied by the rate of emission of *Alnus viridis* at mid elevation from June to mid-October, and the area of *Alnus* stands above 1800 m a.s.l. by the rate of emission at our high *Alnus* plot. Estimated emissions from below and above 1800 m a.s.l. were summed up. This simple approach resulted in a total emission of 130 t N_2O-N per growing season in Switzerland, which represents 1.5% of the total Swiss emissions of this strong greenhouse gas in 2013 (FOEN, 2015). These calculations however, partly underestimate the total annual emissions of *Alnus viridis* because emissions during winter were not taken into account and the areal estimates used disregard the past ten-year expansion of this species. Furthermore, off-plot emissions induced by N losses (through leaching and runoff) add another 0.05–0.13 kg N_2O-N ha^{-1} season^{-1} (EF_5, 0.75% of lost N; IPCC 2006; Bühlmann et al., 2016). By assuming that the rate of emission is the same for the entire year as it is during the growing season, the current area (c. 58 000 ha for 2016) of *A. viridis* in Switzerland is estimated to release about 480 t N_2O-N year^{-1}, which is equivalent to 143 000 t CO_2.

5. Conclusion

From our research, we conclude that the N_2 fixation of *Alnus* species boosts N_2O emissions from soil in otherwise close to natural ecosystems. Warmer conditions did not increase this effect in *Alnus* stands, either in the short-term (seasonal variation in temperature) or in the long term (elevation). The transition from montane pastures to dense *A. viridis* shrubland did not increase the ecosystem C pool. Thus, the massive expansion by *A. viridis* does not represent a C sink. In addition, the CH_4 fluxes remained unaltered in *Alnus* shrubland (preliminary data). Therefore, the substantial release of N_2O by the *Alnus viridis* shrubland represents a continuous net greenhouse gas flux that should be accounted for in inventories of greenhouse gas emissions. Continuous and sustainable land use (grazing, mowing) not only preserves century-old, species-rich montane grassland but also counteracts the release of N_2O induced by *Alnus viridis*.

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Supplementary material

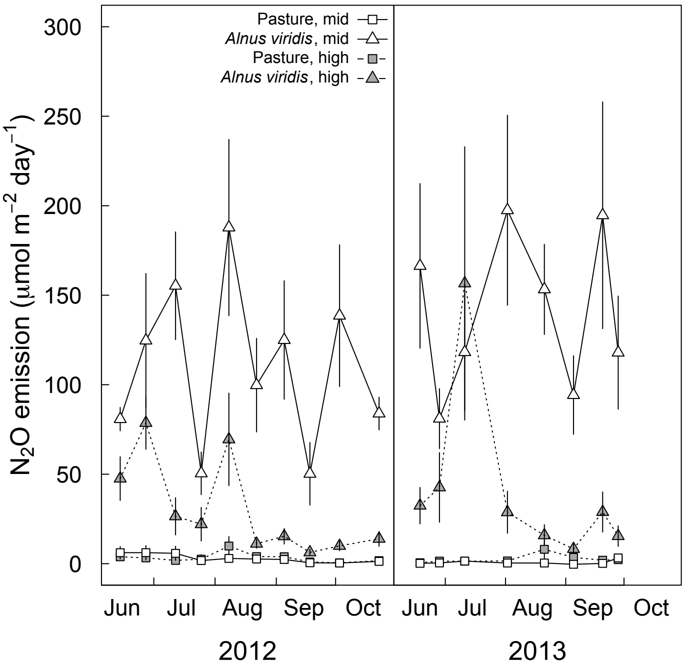

Figure S5.1: Rates of N_2O emission in pastures and *Alnus viridis* at mid and high elevations during the growing seasons 2012 and 2013 (mean ± SE of the raw data).

Table S5.1: Analysis of variance of N_2O emissions dependent on elevation and presence or absence of N_2-fixing vegetation.

Source of variation	Error term	*F*-value	*P*-value
Elevation	Site	$F_{1,4}$=0.01	n.s.
N_2-fixing vegetation	Plot	$F_{1,4}$=8.82	0.04
Elevation × N_2-fixating vegetation	Plot	$F_{1,4}$=0.44	n.s.

Elevation is fitted as continuous variable with levels 1, 2 and 3 for low, medium and high elevation; N_2-fixating vegetation is a two-level factor with the levels control (no *Alnus* present) or *Alnus* (N_2-fixing *Alnus* species present). The analysis uses two error terms: site (*n* = 6) and plot (*n* = 12). Effects of elevation are tested against site, whereas effects related to N_2-fixating vegetation are tested at the level of plots within sites.

Chapter 6

General summary and conclusions

The aim of this thesis was to arrive at an understanding of how increased availability of reactive nitrogen (N_r) caused by atmospheric N deposition or by the dominance of N_2-fixing *Alnus* species influences the N cycle and associated ecosystem properties in close to natural ecosystems.

In Switzerland, atmospheric N deposition represents large N inputs across many close to natural ecosystems and may induce indirect NO and N_2O emissions from soils in these ecosystems. In **chapter 2** of this thesis, we present a model, based on literature data, for the estimation of the indirect emissions in forests, (semi-)natural grasslands and wetlands. In the second part of this thesis **(chapter 3–5),** effects of N_2-fixing *Alnus* on ecosystems, and by N_2O emission on the atmosphere, are addressed. In **chapter 3**, we review the causes of the rapid encroachment of *Alnus viridis* into grassland across the entire Alps, and its effects on biodiversity, ecosystem properties, and economy, and we present management options to reduce the expansion of this shrub. Building on the results from chapter 3, we present a multiyear field study (2009–2012) on the effects of the presence of *Alnus viridis* on N and C pools, and N fluxes, and associated changes in ecosystem properties in comparison to adjacent pastures. This fieldwork was conducted along a West–East gradient in three valleys in central Switzerland (**chapter 4**). Increased temperature and N_r availability is thought to increase N_2O emissions from soils, at least under laboratory conditions. Whether these assumptions hold for field studies was explored in **chapter 5**.

Chapter 2: Induction of indirect N_2O and NO emissions by atmospheric nitrogen deposition in (semi-)natural ecosystems in Switzerland

Bühlmann, T., Hiltbrunner, E., Körner, C., Rihm, B., Achermann, B. 2015.
Atmospheric Environment 103: 94–101

Member states to the international conventions on greenhouse gas emissions (UNFCCC, IPCC, Kyoto Protocol) and on air pollution (UNECE, CLRTAP, Gothenburg protocol) are encouraged to assess the so-called indirect N_2O and NO emissions induced by atmospheric N deposition. However, the approaches to assess these emissions provided by the international organisations are based on agricultural data and may not be suitable for close to natural ecosystems. This causes considerable uncertainties in emission inventories of countries with a large share of close to natural ecosystems, such as Switzerland. The atmospheric N deposition exceeds the critical load for N (CLN) in over 90% of the forest area and raised bogs, in 80% of all fens, and in 30% of all species-rich grassland in Switzerland. Therefore, indirect N_2O and NO emissions from these ecosystems may be substantial.
Based on literature data, we modelled indirect N_2O and NO emissions from forests, grassland and wetland at a high spatial resolution of 100 x 100 m for the years 1990, 2000, 2007 and 2010. We arrived at total national indirect N emissions of 1.61 ± 0.32 Gg N_2O-N and 2.51 ± 0.53 Gg NO-N from close to natural ecosystems in Switzerland in 2010. These emissions account for 21% of the total Swiss N_2O emissions and 10% of the NO_x emissions and are 4.4-times (N_2O) and 17-times (NO) higher compared to results of the approaches of IPCC and EMEP/EEA that are based on data from agricultural systems only. We conclude that the source strengths of close to natural ecosystems are significantly underestimated by these international approaches because emission factors for indirect N_2O and NO emissions of close to natural ecosystems are substantially higher than in agricultural systems. Thus, our approach has the potential to improve the estimate of indirect N emissions. In the year 2015, Switzerland started to account for our approach of estimating indirect N_2O emissions in the national emission inventory (EMIS), and according to the Federal Office for the Environment this new way of assessing national greenhouse gas and air pollutant emissions will be fully established within the next few years.

Chapter 3: *Alnus viridis* expansion contributes to excess reactive nitrogen release, reduces biodiversity and constrains forest succession in the Alps

Bühlmann, T., Hiltbrunner, E., Körner, C. 2014. Alpine Botany 124: 187–191

In this short communication we discuss the rapid expansion of woody taxa into abandoned montane and subalpine grassland in the Alps, focussing on the native shrub *Alnus viridis* (green alder). *Alnus viridis* is spreading at a breath-taking speed due to its ability to symbiotically fix N_2 and its clonal growth. Today, c. 50,000 ha have been overgrown by this shrub in the Swiss Alps, forming monoculture-like, dense shrublands covering entire slopes. Because *Alnus* fixes 20–62 kg N ha^{-1} a^{-1}, it induces large amounts of N_r into these former nutrient-poor grasslands, exceeding the CLN by far. This N load and the dense *Alnus* canopy reduce the biodiversity by more than 50% compared to the former grassland and *Alnus* suppresses species succession towards the natural coniferous montane forests. Even worse, *Alnus* does not secure the overgrown slopes from erosion and unlike forests, it does not protect against avalanches due to its flexible branches. Furthermore, tourists and locals regard traditionally used montane agricultural land as more attractive compared to monotonous shrubland and thus, *Alnus* causes a loss of landscape value. The best way to preserve these montane, cultural landscapes from being overgrown by *Alnus* shrub is sustainable land use. However, regular mowing is labour intensive and expensive. Goats and the traditional sheep breeds Swiss Engadine sheep (‘Engadinerschaf’) and the alpine stone sheep (‘Alpines Steinschaf’) raised in Austria and southern Germany, browse on the bark of woody plants, particularly on that of *Alnus,* and thus are an excellent ‘tool’ to preserve the centuries-old cultural landscape in the Alps or even fight back shrubs and young trees. We recommend increasing the number of animals browsing on bark to reduce the shrub encroachment. To say it in the words of the former ‘manager’ of the ‘Korporation Ursern’ Fredi Russi: ‘Not to take action against the encroachment of these shrubs is most likely the biggest mistake of our generation’ (translated from German to English).

This chapter represents the requested science-policy ‘green paper’ and also led to a ‘factsheet’ by the Swiss Academy of Sciences that created a lot of media attention.

Chapter 4: Shrub expansion of *Alnus viridis* drives former montane grassland into nitrogen saturation

Bühlmann, T., Körner, C., Hiltbrunner, E. 2016. Ecosystems 19: 968–985

The aim of this multiyear field study was to quantify the impacts of the *Alnus* encroachment on N and C pools, N fluxes, and associated changes in soil properties in comparison to not-yet-invaded adjacent montane pastures. The study was conducted along a W-E gradient in three valleys in the Swiss Alps at low (c. 1650 m a.s.l.) and high (c. 1950 m a.s.l.) elevations.

Nitrogen concentration of *Alnus* and understorey plant tissue was generally higher than in pasture plants, indicating an increased N availability in *Alnus* stands. Using ^{15}N isotope analyses, we evidenced that *Alnus* is the source of the increased N_r availability. Overall, *Alnus* stored 570 ± 140 g N m^{-2} and 9800 ± 2100 g C m^{-2} at low elevation (topsoil from 0–30 cm plus above- and belowground phytomass), and 650 ± 90 g N m^{-2} and 9400 ± 700 g C m^{-2} at high elevation. Counter expectations, the total N and C pools were similar in pastures and *Alnus*, because higher plant N and C pools in tissues of *Alnus* were offset by lower soil N and C pools. Therefore, *Alnus* shrubland represents neither a C nor a N sink as compared to pastures.

The soil solution collected by means of ceramic suction cups was highly enriched in nitrate (NO_3^-) under 50-year-old *Alnus* stands at low (2.5 ± 0.4 mg NO_3^--N L^{-1}) and high elevation (0.5 ± 0.2 mg NO_3^--N L^{-1}) during the growing season (June to October). Surprisingly, at low elevation the NO_3^- concentration was twice as high under 25-year-old *Alnus* stands than in the 50-year-old stands, but it was similar in the over 100-year-old stand compared with an 50-year-old stands. Thus, the *Alnus* induced N enrichment persists for more than one century and was highest in young stands. In contrast, NO_3^- concentrations were below 0.1 mg NO_3^--N L^{-1} in pastures in 95% of all samples and resulted in a total leaching measured by means of seepage water collectors (zero-tension lysimeters) of only 0.8 ± 0.1 kg N ha^{-1} at both elevations from June to October. Under 50-year-old *Alnus* stands, N leaching accumulated to 6.9 ± 0.9 kg N ha^{-1} at low and 8.9 ± 1.5 kg N ha^{-1} at high elevation during the same period, with the highest rate of 17.6 kg N ha^{-1} found under 25-year-old *Alnus* at low elevation. When *Alnus* covered more than 20% of the area of a micro-catchment (<1 km^2), nitrate was flushed into streamlets, especially in autumn, when the nitrate availability was highest under *Alnus* stands. Under *Alnus,* the N enrichment and the high NO_3^- leaching reduced the soil pH by

0.35 units and base saturation by 15% in the topsoil compared to adjacent pastures over a period of 50 years.

By comparing the total N input (N_2 fixation and atmospheric N deposition) with the measured N losses (N leaching, N_2O emissions; for details concerning N_2O emissions see chapter 5), we arrived at an overall N balance of -0.5–4.2 g N m^{-2} a^{-1}. However, we found a net loss of -4.6 ± 3.2 g N m^{-2} a^{-1} (mean ± se; se represents the variation between the three sites) at low and -0.2 ± 2.1 g N m^{-2} a^{-1} at high elevation since *Alnus* has overgrown these former grasslands, indicating an overall negative N balance that may result from the N losses, particularly in form of N_2 (and to a smaller extent as NO). We conclude that the amounts of N_r induced by the presence of *Alnus* exceed the CLN of these former nutrient-poor, montane grasslands by far, irrespective of elevation. Given the high N losses, *Alnus* shrublands are clearly N saturated, causing a decline of the water quality in micro-catchments.

Chapter 5: Nitrogen fixation by *Alnus* species boosts soil nitrous oxide emissions

Bühlmann, T., Caprez, R., Hiltbrunner, E., Körner, C., Niklaus, P.A. 2017. European Journal of Soil Science DOI 10.1111/ejss.12457

So far, the effects of increasing temperature and increased N availability on N_2O emissions from soils in close to natural ecosystems are largely unknown, but such indirect emissions may contribute substantially to national greenhouse gas emissions (for indirect N_2O emissions see chapter 2). In this field study, we measured N_2O emissions using a static chamber technique along an elevational gradient of 1.65 km, reflecting a soil temperature gradient of 5.7 K. We always compared non-N_2-fixing vegetation (forests and/or pastures) to N_2-fixing *Alnus* stands (formed by different *Alnus* species), in order to assess the effect of increased N availability. In *Alnus* stands, the N_2O release was clearly higher (on average 1.3 ± 0.2 kg N_2O-N ha^{-1} across all *Alnus* sites) than under non-N_2-fixing vegetation (0.1 kg N_2O-N ha^{-1}), from June to October, indicating that N_2O emissions were indeed induced by the high availability of N_r. Measurements of potential nitrification and denitrification enzyme activity supported our *in situ* N_2O emission measurements. Temperature did not have any influence on N_2O emissions, neither during the growing season nor along the elevational gradient. Only within *A. viridis* stands, we found a decrease in N_2O emissions from mid to higher elevation, which may be explained by the shorter growing season at high elevation (soil temperature was similar due to differences in the exposure). The highest emissions were found at mid elevation under *A. viridis* stands (4.2 ± 0.5 kg N_2O-N ha^{-1} growing season^{-1}) while at the two lower, warmer sites, soils under *A. glutinosa* emitted 0.20 ± 0.05 and 1.65 ± 0.74 kg N_2O-N ha^{-1} during the same period, thus, the specific *Alnus* species seems to have an higher influence on the N_2O emission rates than the environmental conditions. Surprisingly, emissions were still substantial at 1900 m a.s.l. under *A. viridis* (1.2 ± 0.3 kg N ha^{-1} growing season^{-1}). *Alnus viridis* occupies the largest area of the three investigated *Alnus* species in Switzerland and emits a total of approximately 130 t N_2O-N a^{-1}, which represents 1.5% of the total annual Swiss N_2O emissions of 2013, showing a further negative implication of the expansion of this shrubland for the environment (see chapter 3 and 4). We conclude that an *Alnus* induced increase in N availability shows a high potential to enlarge N_2O emissions from soils of close to natural ecosystems.

Conclusions

In summary, these works evidence that the N loads induced by anthropogenic N deposition and by the presence of N_2-fixing *Alnus* species exceed the critical load for nitrogen across many close to natural ecosystems in Switzerland. We conclude, that (1) although atmospheric N deposition has been reduced in Switzerland during the past 25 years, it still induces substantial indirect N_2O and NO emissions from forests, (semi-)natural grasslands and wetlands; (2) continued land use is needed to prevent the centuries-old montane grasslands from being overgrown by the N_2-fixing *Alnus viridis* and thus, to protect this species-rich, valuable landscape from high N loads causing large N losses, and negative consequences on biodiversity, soil, water and air quality; (3) irrespective of elevation, N_2-fixing *Alnus* species drive ecosystems into N saturation with associated high N losses and in case of the species *Alnus viridis* of substantial emission of the strong greenhouse gas N_2O to the atmosphere.

The science-policy part of this thesis had a significant public impact. It alerted stakeholders of the implications of land abandonment on biodiversity, water and air pollution in montane regions, and it helped to improve the national air pollution and greenhouse gas assessments.