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Asymbiotic biological nitrogen fixation in a temperate grassland as affected by management practices

Andreas Keuter*, Edzo Veldkamp, Marife D. Corre

University of Göttingen, Büsgen Institute, Soil Science of Tropical and Subtropical Ecosystems, Büsgenweg 2, 37077 Göttingen, Germany

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ABSTRACT

Estimates of asymbiotic biological N fixation (BNF) in temperate grasslands are few with large variations. In the past six decades, European grasslands have been subjected to intensive management practices and presently it is not known how asymbiotic BNF is influenced by these practices. Our objective was to assess the impact of fertilizer application and mowing frequency on asymbiotic BNF in a Central European grassland. In 2008, we established a three-factorial experiment with two fertilizer treatments (no fertilizer application and combined nitrogen (N), phosphorus (P) and potassium (K) fertilization at 180–30–100 kg ha^{-1} yr^{-1}), two mowing frequencies (cut once and thrice per year) and three sward compositions through the application of herbicides (control, monocot- and dicot-enhanced swards). Three years after the initial sward manipulation, there was no more difference in functional group composition. Between June 2011 and May 2012, we measured in-situ asymbiotic BNF using the acetylene reduction assay, calibrated with $^{15}N_2$-fixation method. Across treatments, asymbiotic BNF rates in the 0–5-cm soil depth ranged from 1.7 (±0.2 SE) kg ha^{-1} yr^{-1} for fertilized plots cut once a year to 5.7 (±2.3 SE) kg ha^{-1} yr^{-1} for unfertilized plots cut thrice a year. Fertilization decreased asymbiotic BNF, suggesting that the potential positive effect of increased soil P levels might have been overruled by the negative effect of increased soil mineral N levels. Intensive mowing stimulated asymbiotic BNF, which was probably due to an increase in rhizodeposition. Our calibration of the acetylene reduction assay with the $^{15}N_2$-fixation method resulted in a conversion factor of 0.61, which largely deviates from the theoretical conversion factor of 3. Furthermore, laboratory incubations under increased soil moisture and temperature conditions overestimated BNF rates compared to in-situ measurements. Thus, laboratory measurements with altered soil moisture, temperature or disturbed soil may lead to strong biases in estimates of asymbiotic BNF. Our results suggest that input of N through BNF may be considerable in temperate grasslands. We conclude that BNF studies should be conducted in-situ and that the acetylene reduction assay should be calibrated against $^{15}N_2$-fixation calibration for reliable estimates.

1. Introduction

Nitrogen (N) is an essential plant nutrient that limits primary productivity in most terrestrial ecosystems (Vitousek and Horwarth, 1991). Although the atmosphere consists of 78% N_2, this N cannot be used directly by plants. Before plants can use N it has to be 'fixed' in a reactive form (e.g. NO_3^- or NH_4^+). There are three major pathways through which N can enter the soil–plant system: atmospheric N deposition, fertilizer addition, and biological N fixation (BNF). During BNF, atmospheric N is reduced to ammonia by microorganisms using the enzyme nitrogenase. In unfertilized terrestrial ecosystems, BNF is typically the most important pathway of N input (Reed et al., 2011).

Since nitrogenase has been isolated from different prokaryotic organisms, asymbiotic BNF is widespread in terrestrial ecosystems. (e.g. Newton, 2007; Haider and Schäffer, 2009; Reed et al., 2011). Asymbiotic N fixing organisms include anaerobic (e.g. Clostridium), facultative aerobic (e.g. Klebsiella, Enterobacter, Bacillus) and aerobic organisms (e.g. Azotobacter). All N fixing organisms use the enzyme complex nitrogenase to catalyze the reduction of dinitrogen to ammonia (e.g. Newton, 2007; Haider and Schäffer, 2009). Nitrogenase is known to be sensitive to O_2 making soil moisture one of the most important drivers for asymbiotic BNF (e.g. Paul et al., 1971; Vlassak et al., 1973; Zechmeister-Boltenstern and Kinzel, 1990; Reed et al., 2011). Since asymbiotic BNF is an energy-

* Corresponding author. Tel.: +49 (0)551 393519; fax: +49 (0)551 393310.
E-mail address: akeuter@gwdg.de (A. Keuter).

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intensive process due to its high ATP requirements, heterotrophic N fixers rely on carbon as an energy source (e.g. Vitousek et al., 2002; Reed et al., 2011). For instance, high rates of BNF have been reported for straw amended soils (Paul et al., 1971) or after the addition of artificial root exudates (Bürgmann et al., 2002).

In the past few decades, human activity has more than doubled the global reactive N input through production of chemical N fertilizers, cultivation of legumes in agriculture, and production of N-oxides during combustion of fossil fuels (Vitousek et al., 1997). Whereas the increase in reactive N input through fertilizer addition and atmospheric N deposition have been reviewed extensively (e.g. Galloway et al., 2008), studies on BNF are limited, unequally distributed among biomes and with large variations in rates (Reed et al., 2011). In many ecosystems, symbiotic N fixation is the dominant pathway for BNF. However, asymbiotic BNF fixation by free-living microorganisms may be an important pathway of N input in ecosystems where no or only few leguminous species are present. Existing data suggest that asymbiotic BNF may be dominant in temperate grassland ecosystems with rates ranging from 0.1 to 21 kg N ha^{-1} yr^{-1} with a mean of 4.7 kg N ha^{-1} yr^{-1} from 13 sites (Reed et al., 2011).

Most studies that measure BNF use the acetylene (C_2H_2) reduction assay. This method is based on the discovery that nitrogenase, the enzyme responsible for BNF, also reduces C_2H_2 to ethylene (C_2H_4) (Hardy et al., 1968). Although nowadays BNF can be measured directly using $^{15}N_2$ gas, the C_2H_2-reduction assay is still widely used because it is a low-cost and quick method with a low detection limit. In many published BNF estimates, a theoretical conversion factor has been used in the calculation of N fixation rates from C_2H_2-reduction rates. However, cross calibrations with the $^{15}N_2$-fixation method have shown that conversion factors can deviate substantially from the stoichiometrical value of 3 mol C_2H_4 per mol N_2 (see e.g. Nohrstedt, 1983a). Furthermore, many asymbiotic BNF estimates are based on laboratory incubations with altered temperature and/or moisture conditions, which can strongly affect asymbiotic BNF rates. Many previous studies also used disturbed soil samples, which probably altered the mineral N and oxygen concentrations in the soil and hence influenced the activity of the N- and oxygen-sensitive nitrogenase enzyme. We found only one in-situ study in a temperate grassland where asymbiotic BNF was estimated on undisturbed soil samples and where the conversion factor of the acetylene reduction assay was calibrated against the $^{15}N_2$-fixation method (Skujins et al., 1987). This study was conducted in the subalpine zone of Utah, USA which makes it difficult to compare with Central European grasslands.

Apart from the large uncertainty surrounding the magnitude and significance of asymbiotic BNF, there is also a dearth of information on how ecological controls and agricultural management practices affect asymbiotic BNF (Cleveland et al., 1999; Vitousek et al., 2002). This lack of knowledge restricts our ability to predict future changes in asymbiotic BNF resulting from changes in land-use management and environmental conditions (Reed et al., 2011). N and phosphorus (P) can influence nitrogenase activity. Soil mineral N can inhibit nitrogenase (e.g. Yoch and Whiting, 1986) while P can be a limiting nutrient since asymbiotic BNF has high ATP requirements (e.g. Eisele et al., 1989; Vitousek et al., 2002; Reed et al., 2007, 2011). Apart from N and P, molybdenum (Mo) availability may be an important driver for asymbiotic BNF since Mo is the central element of Mo-nitrogenase, the most efficient N fixing enzyme (e.g. Vitousek et al., 2002; Reed et al., 2011). Positive effects of Mo-availability on BNF have been reported by Barron et al. (2008) for tropical forest soils.

In the last half century, European grasslands have been subjected to agricultural intensification (i.e. increased mowing frequency and fertilizer input); however, no studies have been conducted on the combined influence of mowing and NPK-fertilizer application on asymbiotic BNF. Studies in grasslands in the UK (Lockyer and Cowling, 1977) and USA (Vlassak et al., 1973) reported negative effects of N fertilization on asymbiotic BNF, which was attributed to inhibitory effects of high soil mineral N concentrations on nitrogenase (Yoch and Whiting, 1986). Furthermore, P is frequently discussed as a critical nutrient since asymbiotic BNF has a high energy requirement which is provided by adenosine triphosphate. A study in an infertile grassland ecosystem in Colorado, USA reported a strong positive effect of increased P concentrations through P fertilization on asymbiotic BNF (Reed et al., 2007). Since N and P have contrasting effects on asymbiotic BNF, it has been suggested that N:P ratios may be a better predictor of asymbiotic BNF rates rather than N or P concentrations alone (Eisele et al., 1989). However, in soil crusts in a native black grama grassland in New Mexico, USA, neither P concentrations nor N:P ratios correlated with asymbiotic BNF rates (Hartley and Schlesinger, 2002). They suggested that soil mineral N and available carbon (C) rather than P or N:P ratios controlled asymbiotic BNF. We did not find any study on the effect of potassium (K) on asymbiotic BNF. K is an essential nutrient and deprivation of K may lead to inhibition of nitrogenase activity (Alahari and Apte, 1998). Furthermore, if K is limiting, K fertilization can increase plant productivity which, in turn, may increase C input into the soil through root exudation. Finally, K application may temporarily lead to increases in salt concentrations which may affect asymbiotic BNF through osmotic effects (Fernandes et al., 1993).

Mowing frequency may potentially affect asymbiotic BNF in grasslands since plant defoliation increases rhizodeposition, providing available C for soil microorganisms (e.g. Holland et al., 1996), which may have a positive effect on the energy-demanding asymbiotic BNF. In a laboratory experiment, an increase in asymbiotic BNF was observed following the addition of artificially prepared root exudates to soil samples (Bürgmann et al., 2005). If mowing stimulates asymbiotic BNF through increasing rhizodeposition, we would expect that this would be reflected in increasing soil microbial biomass which could assimilate the high amounts of C released into the rhizosphere (Guitian and Bardgett, 2000). Since asymbiotic BNF is an energy-intensive process, C input may increase asymbiotic BNF by providing energy for heterotrophic N fixers.

Here, we present the results of a study that aimed to assess the impact of fertilizer application and mowing frequency on asymbiotic BNF in a temperate grassland. We tested the following hypotheses: 1) combined application of N, P and K at a standard rate for grasslands in Germany (180–30–100 kg NPK ha^{-1} yr^{-1}) will decrease asymbiotic BNF; 2) intensive mowing will increase asymbiotic BNF due to a resulting increase in rhizodeposition of C which will be reflected in a larger soil microbial biomass.

2. Material and methods

2.1. Site description

Our study (which is part of the grassland management project "GRASSMAN") was conducted at a moderately species rich, permanent grassland site in the Solling uplands, Lower Saxony, Germany (51°44′53″N, 9°32′42″E, at 490 m above sea level). This site, which presently belongs to the experimental farm Relliehausen, has been used for grazing and hay production for at least 100 years (Geological Map of Prussia 1910, based on the topographic inventory of 1896; topographic maps of Sievershausen and Neuhaus/Solling 1924, 1956 and 1974). During the last 50 years, the site was used for cattle grazing, received moderate fertilizer applications (80 kg N ha^{-1} yr^{-1}), and was occasionally limed and overseeded