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S. I. BIOCHAR

Potential nitrogen fixation changes under different land uses as influenced by seasons and biochar amendments

Agota Horel[1] · Imre Potyo[1] · Tibor Szili-Kovacs[1] · Sandor Molnar[1]

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Abstract
Soil nutrient dynamics, potential biological nitrogen fixation (BNF) changes, and their relations were studied using four land use types. Further, we investigated BNF changes in the presence of biochar in soils. Soil samples were collected from arable, vineyard, grassland, and forest soils during four seasons, and analyzed for abiotic contents of total nitrogen, NH_4^+-N, NO_3^--N, ammonium lactate (AL)-soluble K_2O, P_2O_5, and soil organic carbon (SOC) concentrations. Potential N_2 fixation was measured as ethylene (C_2H_4) production from acetylene (C_2H_2) reduction (ARA). The study focused on the changes in ARA when different types of biochars (T600, T650, and T700) were applied to soil samples in different amounts (0, 0.5, 2.5, and 5.0% wt wt^{-1}) under laboratory conditions. We found strong correlations between soil chemical parameters and ARA values, especially in the case of soil pH, total N, SOC, and P_2O_5 contents. In the case of arable soil, the ARA measurements were up to 227 times higher compared to grassland and forest samples. Biochar application affected N_2-fixing microbial responses among land use types, most notably decreases in arable lands and forest soils. We found that a high amount of biochar added to the soils can greatly suppress N_2-fixing activities. Our results highlight the strong relationship between soil nutrient changes and the intensity of anthropogenic influence.

Keywords Arable · BNF · ARA · Forest · Grassland · Vineyard

Introduction

Anthropogenic activities affect soil nutrient dynamics including nitrogen cycling in agroecosystems. Long-term cultivation and management of a given land use may result in significant alterations in soil nutrient cycles and microbial community compositions Ye et al. 2009). Different tillage practices or addition of fertilizers to soils can further modify soil structures and microbial communities that consequently influence soil biochemical processes (Mijangos et al. 2006). Land use and plant types in a given area can determine the requirement for additional nutrients that need to be added to the soil for better crop growth and yield (Fageria 2001). Loss of plant nutrients can occur in different ways when applied in excess. Nitrogen mostly leaves the soil matrix through hydrological and biogeochemical processes, e.g., leaching, ammonia volatilization, or by gaseous loss through nitrification and denitrification (Reddy et al. 1984). In agricultural croplands, such as winter wheat or grapes, fertilizer application and soil tillage are common practices to ensure high crop yield or better water infiltration (Kanwar et al. 1988), while in the case of grassland and forest soils, the anthropogenic impact is less disruptive. Therefore, for a better understanding of the complexity of land use systems on soil nutrient dynamics in a given area, different land uses should be investigated.

The fixation of N_2 is a very important path to enhance the soil nitrogen availability in many ecosystems. When nitrogen is present in soils in limited supply, the rates of BNF can increase. N_2-fixing bacteria, called diazotrophs, can convert N_2 gas to ammonia using nitrogenase enzymes, which provide available nitrogen for plants (Santi et al. 2013). N_2-fixing bacteria are responsible for approximately 90×10^{12} g biologically fixed nitrogen per year in the case of agricultural land, and an additional 50×10^{12} g biological nitrogen fix per year for forest and non-agricultural lands, globally (Bezdicek and

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✉ Agota Horel
horel.agota@agrar.mta.hu

[1] Institute of Soil Sciences and Agricultural Chemistry, Centre for Agricultural Research, Hungarian Academy of Sciences, Herman O. St. 15, Budapest 1022, Hungary

Kennedy 1998). N_2 fixation is altered by human activities (Vitousek et al. 1997) as fertilizer application to cultivated soils can greatly influence the microbial density and diversity of the soil (Maharning et al. 2009). Several types of free-living N_2-fixing bacteria are present in soils, either anaerobes such as *Desulfovibrio* and *Clostridium* spp., or aerobe phototrophs, e.g. *Cyanobacteria*, or aerobe heterotroph *Azotobacter* spp.

In recent years, soil additives such as biochar are getting extra attention as they might help crop production in agricultural fields and also can mitigate negative effects of greenhouse gases originating from the soils. Recent studies on the effect of biochar application to soils and its rates to fix N_2 vary in literature. While there are several studies investigating symbiotic biological N_2 fixation (BNF) response to biochar amendment to soils (Rillig et al. 2010; Rondon et al. 2007), non-symbiotic or free-living N_2-fixing bacterial communities' responses are less studied (Atkinson et al. 2010). It has been reported that biochar can increase BNF in agricultural soils such as soils planted with red clover (Mia et al. 2014) or common beans (Rondon et al. 2007). Biochar can also increase the alkalinity of acidic soils, creating more favorable conditions for N_2-fixing bacteria (Rondon et al. 2007), though a decrease in BNF rates at high biochar amendments might also occur. Our current knowledge on the direct and indirect effects of biochar application to soils on various nitrogen cycling processes, such as N_2 fixation or nitrogen mineralization, is still lacking (DeLuca et al. 2009); therefore, soil and biochar-specific studies should be conducted prior to soil additive use, especially in the case of soils sowed with non-legume plants.

In this paper, we aimed at assessing nutrient and ARA changes in four land use types (arable, vineyard, forest, and grassland) during different seasons, where the land uses have similar soil structures. Since the intensity of anthropogenic influence on nitrogen cycling processes is still less known with new soil additives being developed to promote agricultural productivities, we investigated how different types and amount of biochar addition influence the ARA rates of these land uses. We hypothesized that (i) soil nutrient and ARA changes will differ considerably among land use types and seasons, (ii) disruptions in soil chemical parameters can negatively affect potential BNF rates, and (iii) different amounts and types of biochar amendments to soils influence the rates of ARA differently.

Materials and methods

To address our hypotheses on how anthropogenic activities alter soil biotic and abiotic processes, we performed our experiments in two ways. The present study included field trials investigating soil chemical changes with special emphasis on nitrogen fixation potentials over time under different land uses. Later, we supplemented our findings with data retrieved from a laboratory experiment where we could closely monitor microbial response to biochar amendments focusing on changes in ARA, using soil samples collected from the field.

Soil sampling, site information, and soil chemical analyses

Soil samples (Luvisol, WRB) were collected from (i) a tilled arable soil sowed with winter wheat (46.92649° N, 17.68246° E), (ii) a vineyard (*Vitis vinifera*; 46.9166° N, 17.68976° E), (iii) a grassland (meadow; 46.91232° N, 17.69754° E), and (iv) a forested area (oak and maple mix; 46.91283° N, 17.69723° E). All soil samples were collected from a small agricultural catchment located in Balaton Uplands, Hungary. The four land use types were chosen as they represent different time scales and levels of human impacts on soils and also characterize large portions of many agricultural lands at a given area. Arable land experiences annual plowing and frequent fertilizer and herbicide applications, and crop rotation (e.g., winter wheat, triticale). Vineyards have the same plant every year receiving in-row plowing, with frequent fertilizer applications. Grasslands have only hay harvesting, but no tillage or chemical amendments. Soil from the forest floor receives minimal anthropogenic impact, as no tree cutting was performed nor were any dead trees removed in recent decades.

All samples were taken from the upper 2–12 cm soil layer by sample corer at three sampling points per land use evenly distributed along an approximately 15-m-long transect line. Samples were collected 3 months apart in February, May, July, and November, representing all four seasons, respectively. All soil samples in vineyards were collected from in-row plowing area. Soil samples were homogenized and analyzed for total nitrogen content, NH_4^+-N, NO_3^--N, K_2O (AL soluble), P_2O_5 (AL soluble), soil organic carbon (SOC), electrical conductivity, and pH_{H2O}. NH_4^+-N and NO_3^--N values were obtained based on KCl extraction and stream distillation technique. SOC contents were measured by wet digestion using the Tyurin method. The total nitrogen was determined using the modified Kjeldahl method (ISO 11261:1995). K_2O and P_2O_5 measurements were done using an inductively coupled plasma optical emission spectrometry (Quotation ICP-OES, Ultima 2) after ammonium lactate extraction (AL). Most of these measurements in the present study were chosen as basic indicators to analyze soil chemical changes and nutrient dynamics, so we could get a more complete picture of carbon and nitrogen cycling at the investigated sites. In addition, we also measured $CaCO_3$ contents using Scheibler calcimeter for the arable and vineyard soils. Soil element concentrations are reported as mg kg^{-1} dry weight soil.

Particle size distribution was determined using the sieve-pipette method, where arable, vineyard, grass, and forest soil had 10.4 ± 0.84, 12.05 ± 1.32, 22.67 ± 0.81, and $15.86 \pm$

0.31% sand content (2–0.05 mm), 44.8 ± 1.14, 36.18 ± 2.70, 39.85 ± 2.78, and 54.95 ± 0.53% silt (0.05–0.002 mm), and 44.78 ± 0.31, 51.76 ± 2.70, 37.48 ± 2.26, and 29.19 ± 0.30% clay (< 0.002 mm) content, respectively.

Biochar types used in the experiment

The chemical characteristics of the three types of biochar prepared at three pyrolysis temperatures (T600, T650, and T700) used in the present study are shown in Table 1. The three biochar types were manufactured at factories provided with European Biochar Certificates. According to the manufacturers' information, biochar T600 was made from cellulose fibers and grain husks using Pyreg technology at 600 °C; biochar T650 was made from woodchips with Pyreg technology at approximately 650 °C; and biochar T700 was made from woodchips using the Schottdorf system at approximately 700 °C. The biochars were analyzed for different nutrient concentrations such as NH_4^+-N, NO_3^--N, total nitrogen content, P_2O_5, K_2O, and pH using the same standard chemical techniques described for soil samples.

Measuring potential N_2-fixing bacterial activities using gas chromatography with flame ionization detector

Soil samples for all four land use types were analyzed for potential BNF rates calculated from acetylene reduction. The effects of different biochar types and amounts were also investigated on samples collected during spring (May). Potential nitrogen (N_2) fixation or BNF was measured as ethylene (C_2H_4) production from acetylene (C_2H_2) reduction (ARA) (Welsh et al. 1996). From the homogenized soil, 10 g dry weight soil was added to triplicate 27-ml serum vials. The different biochar amounts (0, 0.5%, 2.5%, and 5.0%) of T600, T650, and T700 were added to the vials prior to the addition of 4 ml of glucose solution (50 g l^{-1}) in distilled water. Control samples had no biochar addition. Vials then were capped and placed in an incubator for 24 h at 25 °C to increase the number of heterotrophic nitrogen-fixing bacteria in the soil. All samples but controls received 10% (*v*/*v*) of C_2H_2, added to the headspace of the vials, and incubated for an additional hour. Samples without C_2H_2 were used to develop a baseline for occasional ethylene production in the soil samples, with the values deducted from the measured concentrations. After 1 h incubation, the samples were measured for ethylene production. Production of C_2H_4 from the reduction of C_2H_2 as a substrate analog of N_2 was measured using a FISONS 8000 gas chromatograph with flame ionization detector (GC-FID). All rates and fluxes pertaining to nitrogen species are expressed on a nitrogen atom basis.

The GC-FID oven temperature was held constant at 80 °C, while the detector temperature was held at 100 °C during measurements. The carrier gas was nitrogen with a constant flow of 30 ml min^{-1} (170 kPa). The GC column Porapak N (80–100 mesh) was 2–3 m in length with 2.1 mm internal diameter and 3.2 mm outer diameter. Gas samples (125 μl) were manually injected into the GC. Ethylene standards (10 mg kg^{-1}) were used to quantify measurements and to qualify instrument reliability after being in use for a longer period of time. Ethylene concentration was calculated from the peak area provided by the Clarity software using calibration gas.

Statistics

The factors of land use types (arable, vineyard, grassland, or forest), biochar types, and amounts (T600, T650, or T700; 0, 0.5, 2.5, or 5.0% by weight), as well as their interactions in relation to non-amended soils (0 or control), were analyzed using one- or two-way analysis of variance (ANOVA) followed by a post hoc Tukey HSD test. Residuals were checked for normal distribution and data were transformed (Box-Cox transformation) where necessary. All statistical calculations were performed using the software package R (Version 2.15.2). Statistical significance of the data sets was determined at $p < 0.05$ and $p < 0.01$.

Q1 **Table 1** Chemical characteristics of the three biochar types used in the experiment ($n = 3$; ± SD)

Biochar type	pH-H_2O	Al-K_2O (mg kg^{-1})	Al-P_2O_5 (mg kg^{-1})	Total N (%)	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	TOC (%)
T600	10.3 ± 0	13,570.3 ± 59.1	5031.1 ± 32.6	1.01 ± 0.1	1.86 ± 0	n. d.	47.3[a]
T650	9.6 ± 0	4407.5 ± 0.9	463.2 ± 2.8	0.84 ± 0.03	1.81 ± 0.07	n. d.	45.7[a]
T700	9.5 ± 0.04	1868.2 ± 50.9	260.4 ± 6.7	0.24 ± 0.01	1.68 ± 0	n. d.	38.8[b]

T600, T650, and T700 represent biochar pyrolysis temperatures of 600, 650, and 700 °C

TOC total organic carbon values, *n. d.* not detectable

[a] Data were based on manufacturers' certificate

[b] Soil organic carbon (SOC; %)

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Results

Changes in soil nutrients over time

Soil samples were collected at four seasonally distinguishable periods to investigate the changes in soil nutrients and BNF rates over time (Table 2, Fig. 1). Analyzing the total nitrogen amount of the four land use types in winter soil samples, arable soil showed significant differences ($p < 0.05$) compared to forest and grassland soils, while vineyard data showed significant differences compared to forest (Table 2). When investigating spring samples, we also observed significant differences between vineyard's and grassland's total N values. These differences however diminished during summer and fall sampling periods, where none of the land use types' total N showed statistically significant differences compared to each other (Table 2).

Soil samples collected during winter and spring showed significant differences between arable or vineyard soils' SOC contents compared to grassland or forest soils ($p < 0.04$), while SOC values of the land use types showed no significant differences in summer or fall samples ($p = 0.1949$ and $p = 0.1446$, respectively; Table 2).

Fertilizer addition to agricultural lands is a common practice worldwide to achieve better crop yield; however, it also affects the soils' nitrogen forms. Total N contents were the highest in samples collected in spring for all land use types except in the case of arable soil, where summer samples had the highest amount. We observed a decrease in total N concentrations during winter in all land use types. However, total N data in arable soil only showed significant changes when comparing winter samples to other seasons ($p < 0.04$). In the case of grassland, similar results were observed as in the arable land when analyzing samples collected in spring ($p < 0.02$). In terms of vineyard or forest soils, we did not observe any significant differences in total N concentrations seasonally.

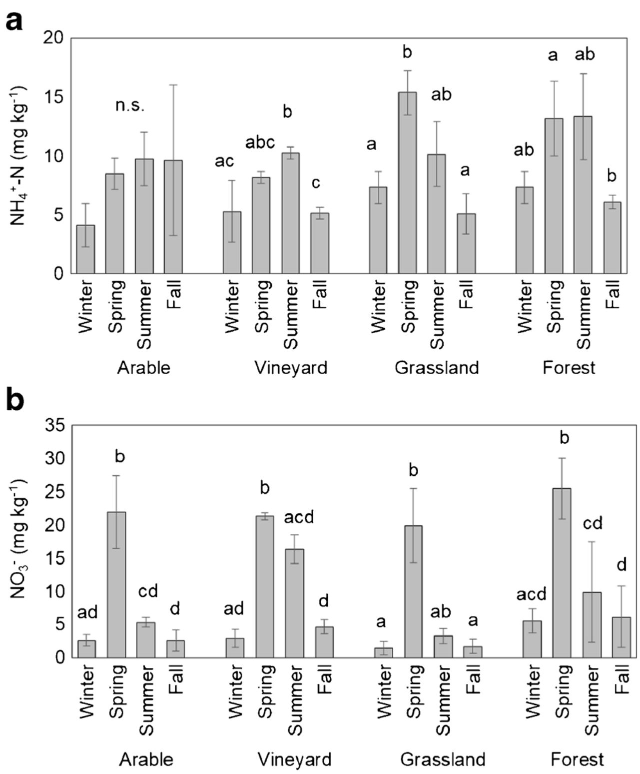
The trend in changes of SOC over time was similar to changes in total N, all land use soil samples (but arable) showed a peak in its SOC amount during spring and slowly decreased toward the end of the year, while arable soil had similar SOC among seasons with the highest observed in fall months (Table 2). In terms of arable and forest soils, the SOC values did not show significant differences over time ($p > 0.05$). Vineyard SOC values measured in fall soil samples showed significant differences compared to other seasons, while winter, spring, and summer data had no significant differences. In the case of grassland, spring soil samples had significantly greater SOC compared to other seasons' data ($p < 0.05$). Overall, we found significant differences between the four land use types, mainly arable and vineyard compared to grass or forest soils, signifying the connections between human impact and soil nitrogen and carbon stocks.

In general, when analyzing NH_4^+-N and NO_3^--N data, we did not find any statistically significant differences between land use types in any of the sampling periods (Fig. 1); however, we observed that NH_4^+-N and NO_3^--N concentrations were the highest during the spring and summer months.

Forest soils showed the lowest K_2O concentrations (124.51–226.67 mg kg^{-1}) with significant differences compared to the other land use types ($p < 0.027$; Fig. 2a). Vineyard samples had the highest K_2O concentrations

t2.1 **Table 2** Soil chemical characteristics of the four land use types at different sampling periods ($n = 3$; ± SD)

	Sampling times	Land use types	$pH_{(H2O)}$		Total N (%)		SOC (%)		$CaCO_3$ (%)		EC (mS cm^{-1})	
t2.2												
t2.3	February	Arable	7.98	± 0.02	0.21	± 0.04	1.76	± 0.04	20.63	± 0.62	0.25	± 0.01
t2.4	Winter	Vineyard	8.01	± 0.02	0.23	± 0.02	2.07	± 0.11	31.92	± 3.54	0.29	± 0.02
t2.5		Grassland	6.54	± 0.16	0.34	± 0.04	3.08	± 0.39	0	–	0.3	± 0.05
t2.6		Forest	6.11	± 0.37	0.43	± 0.07	3.86	± 0.58	0	–	0.2	± 0.08
t2.7	May	Arable	7.9	± 0.02	0.23	± 0.01	1.75	± 0.20	20.04	± 0.59	0.25	± 0.02
t2.8	Spring	Vineyard	7.89	± 0.01	0.26	± 0.01	2.29	± 0.15	29.8	± 4.89	0.33	± 0.01
t2.9		Grassland	6.85	± 0.15	0.49	± 0.06	4.24	± 0.48	0	–	0.48	± 0.09
t2.10		Forest	5.91	± 0.49	0.51	± 0.05	4.62	± 0.20	0	–	0.27	± 0.11
t2.11	August	Arable	7.85	± 0.04	0.24	± 0.01	1.6	± 0.14	20.35	± 0.28	0.26	± 0.01
t2.12	Summer	Vineyard	7.8	± 0.04	0.24	± 0.02	2.09	± 0.10	31.52	± 4.48	0.37	± 0.02
t2.13		Grassland	6.91	± 0.17	0.27	± 0.04	2.14	± 0.45	0	–	0.31	± 0.08
t2.14		Forest	5.52	± 0.12	0.37	± 0.15	3.56	± 1.68	0	–	0.15	± 0.05
t2.15	November	Arable	7.78	± 0.05	0.24	± 0.01	1.8	± 0.05	21.09	± 0.09	0.25	± 0.01
t2.16	Fall	Vineyard	7.87	± 0.02	0.21	± 0.02	1.68	± 0.19	31.46	± 8.20	0.27	± 0.01
t2.17		Grassland	6.86	± 0.20	0.23	± 0.04	2.06	± 0.19	0	–	0.24	± 0.04
t2.18		Forest	5.38	± 0.14	0.3	± 0.09	2.86	± 0.93	0	–	0.09	± 0.03



Q2 **Fig. 1** Soil inorganic nitrogen concentration changes in the various land use types. **a** NH_4^+-N. **b** NO_3^--N. Nutrient concentrations are on a dry soil mass basis ($n = 3$; ± SD). Statistically significant differences are indicated by different letters within land use types. n.s. not significant

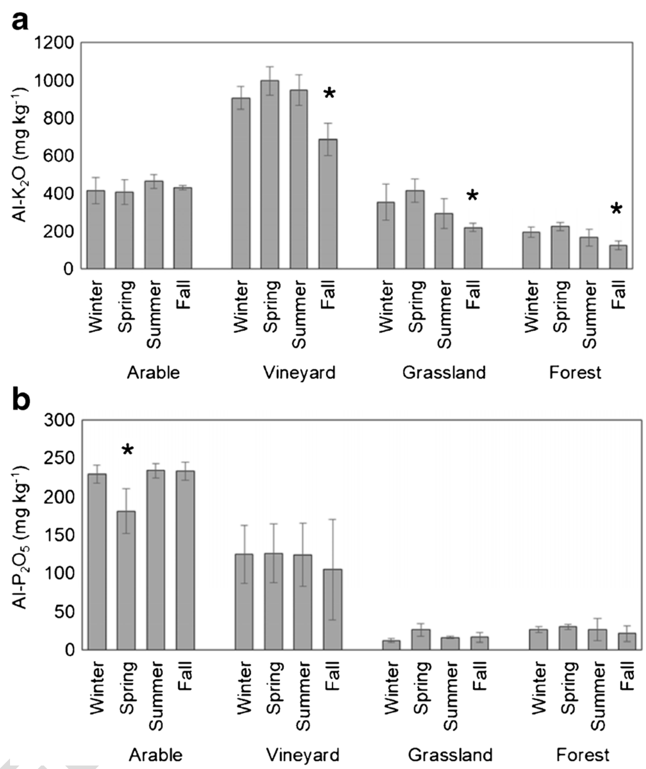


Fig. 2 Soil inorganic nutrient concentration changes in the various land use types. **a** K_2O. **b** P_2O_5. Nutrient concentrations are on a dry soil mass basis ($n = 3$; ± SD). * represents significance level of $p < 0.05$ within land use types

(687.12–997.67 mg kg^{-1}), which also were significantly different compared to the other land uses ($p < 0.001$). In terms of P_2O_5 contents, arable land showed the highest values (181.33–233.76 mg kg^{-1}), resulting in substantial differences compared to all other land use ($p < 0.004$), while the lowest (12.24–26.17 mg kg^{-1}) were observed in the case of grass soil (grassland compared to arable $p < 0.001$, and vineyard $p = 0.005$; Fig. 2b), but not in the case of the forest samples.

Changes in ARA during different seasons

Changes in ARA are shown in Fig. 3 for the different land use types. Arable soil showed the highest ARA (24.10 nmol C_2H_4 g^{-1} soil h^{-1}) among all land use types in all four seasons, while the lowest amounts were the most noticeable in the case of forest soils (0.11 nmol C_2H_4 g^{-1} soil h^{-1}). Arable and vineyard soils showed similarly high ARA values, and the forest similarly low ARA among seasons, while grassland samples showed seasonal effects on ARA. Grassland had the highest N_2-fixing potential during summer, and it continuously decreased in fall, winter, and somewhat in spring.

Because of the high ARA values in arable land, we expected to see statistically verified differences as well. After comparing the data, we found that winter, summer, and fall arable soil samples had significantly higher ARA ($p < 0.003$, $p < 0.014$, and $p < 0.008$, respectively) compared to other land use types, while C_2H_2 reduction of spring arable soil samples differed significantly only in the case of grassland and forest soils ($p = 0.031$). Seasonal changes did not result in major ARA changes in arable, vineyard, and forest soils. Summer ARA rates in grasslands were significantly different compared to the other seasons ($p = 0.047$; Fig. 3).

Relationships between different soil chemical properties and ARA

As chemical properties of a specific soil can greatly influence its biological reactions, we explored the connections between soil nitrogen, carbon, phosphor, or pH changes and acetylene reductions (Fig. 4). We found strong correlations between ARA and total N, SOC, soil pH, or P_2O_5 contents (Fig. 4). We did not find strong correlations with ARA values when investigating soil NH_4^+-N and NO_3^--N concentrations ($p = 0.26$ and $p = 0.66$, respectively). Our results showed that with increasing soil total N or SOC concentrations, the N_2 fixation potential decreased. In the case of P_2O_5 data, we found that at low concentrations, the acetylene reductions were low as well, and increasing P_2O_5 contents resulted in increased ARA values. In the case of soil pH, we observed that acidic conditions resulted in low ARA, while around pH 8, the ARA were

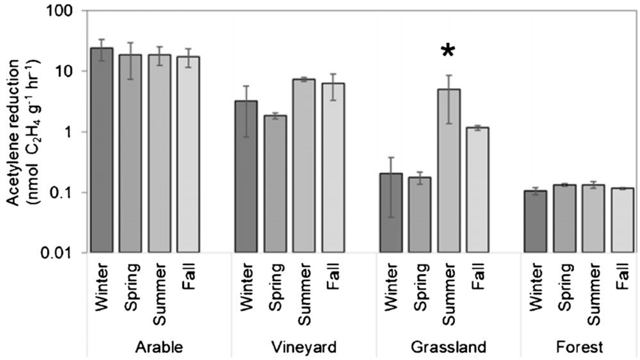


Fig. 3 Acetylene reduction activity (ARA) of soil samples of the varying land use types collected during different seasons, based on C_2H_4 production rate. * represents significance level of $p < 0.05$ within land use types; $n = 3$, ± SD

no longer inhibited and the values were influenced by other soil parameters (Fig. 4d).

Effects of biochar application on soil N_2-fixing potentials

Biochar application affected N_2-fixing microbial activities differently among land use types. The type of the biochar used in the experiment was also an important factor in the changes observed in ARA. Based on data retrieved from spring soil samples, we observed the highest ARA in the case of arable land with up to 18.42 ± 11.1 nmol C_2H_4 g^{-1} h^{-1} followed by vineyard soils, where soil amendment frequently includes the use of fertilizer (Fig. 5a, b). In the case of grassland and forest soils, the ARA values were considerably lower compared to arable or vineyard soils, ranging between 0.15 and 0.25 nmol C_2H_4 g^{-1} h^{-1} (forest 5.0% T700 and grassland 0.5% T700, respectively; Fig. 5c, d).

When examining the type of biochars used in the experiment, we found that T600 addition to soils provided higher BNF rates in most cases compared to the other two types of biochars (Fig. 5). Ethylene production from soils amended with T650 or T700 biochars showed very similar microbial responses (Fig. 5), showing that different biochars can extensively influence microbial activities. Comparing to control, we also found significant difference in ARA for the arable soil compared to the T650- or T700-amended soils ($p < 0.001$), except for 0.5% T650 vineyard ($p = 0.0511$) and 5.0% T700 vineyard soils ($p = 0.0594$). T600-amended samples showed a slight increase in N_2 fixation rates in arable soils compared to control treatments; however, significant differences were mainly observed in the 0.5% T600-amended arable soils compared to the other treatments. Although these differences were significant, it is also worth noting that all p values were above 0.0485.

When investigating samples with the lowest amount of biochar application (0.5%) among the different types of biochars, we only observed significant differences between

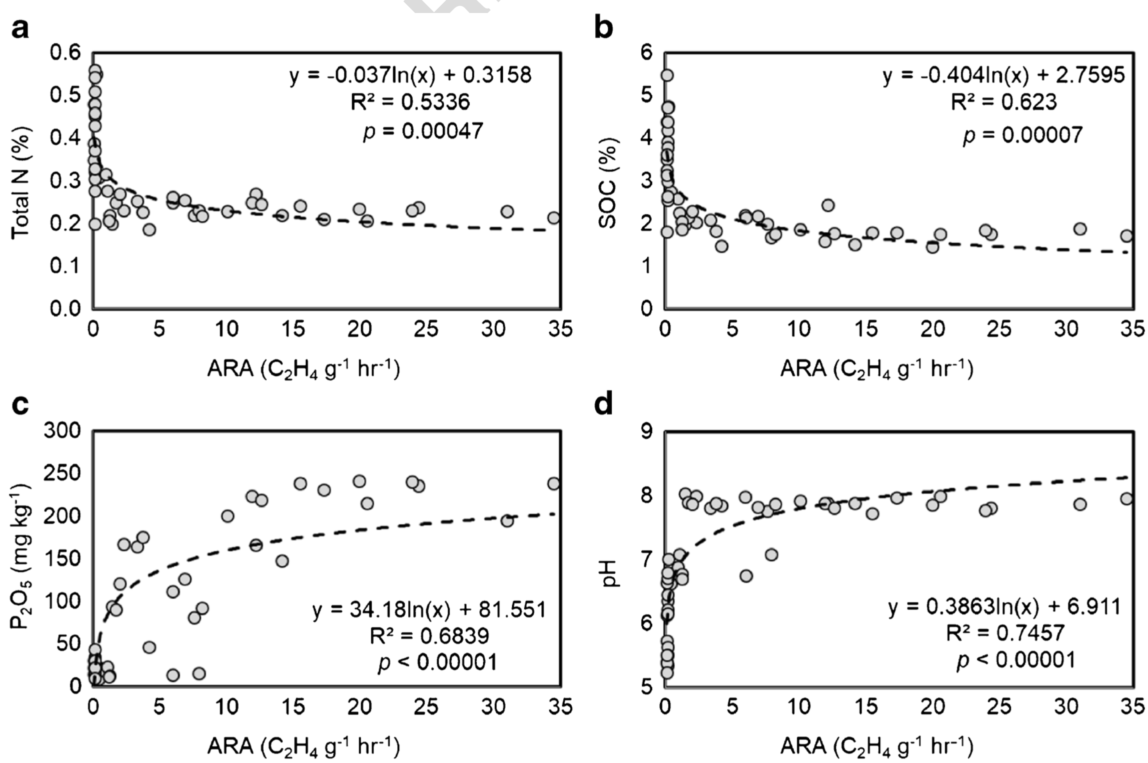


Fig. 4 Relationships between soil chemical properties of **a** total N, **b** SOC, **c** P_2O_5, **d** pH, and acetylene reduction activity (ARA) measured for the different land uses. $n = 48$

T600-amended arable soil, mostly grassland, and forest soils ($p < 0.02$; Fig. 5), while all other low biochar amounts did not result in significant differences in ARA values. When 2.5% biochar was added to the soil samples, all ARA reduced to a level, where substantial changes within land use types and biochar types ($p = 0.057$) could not be observed. The 5.0% biochar addition resulted in a major decrease in ARA for all soils regardless of land use types ($p = 0.028$).

Discussion

In the present study, potential BNF rates, as analog to acetylene reduction activities, showed a substantial increase in its values during summer and fall compared to winter or spring. This increase was the most noticeable in the grassland soil samples. The increase in BNF rates could be related to elevated temperatures during summer and fall seasons, as one of the major differences between sampling sites was the seasonal temperature shift. Although biological N_2 fixing has shown significant changes with varying temperature and soil moisture (Belnap 2003; Horel et al. 2014), in the present study, the other sampling sites' BNF rates were less pronounced when considering temperature differences only. Our finding suggests the diversity of microbial communities and/or densities among sites rather than climatic factors. Many agricultural sites during hot summer with low soil moisture and cold winter temperatures can result in suppressed nitrogenase and metabolic activities of the microbial communities, leading to low N_2 fixation values (Belnap 2003). In the present study, winter temperatures were unusually high for the area with below average precipitation amount. However, during summer and fall months, several rain events took place, which could cause the increase in microbial density, hence resulting in higher BNF potentials. Soil moisture differences can influence microbial responses to available carbon source at a given area, which further can be influenced by different vegetation succession stages (Surda et al. 2015). Leaf interception, especially in the case of forest, can significantly reduce the throughfall amount, which in summer months can result in significantly lowered soil moisture contents. In the present study, summer soil moisture contents of forest soil were 55.9% less than when compared to spring samples; however, the N_2 fixation potentials were not affected by these differences among seasons.

Chemical characteristics of the investigated soil can further influence BNF rates. pH ranges of soils can affect N_2-fixing rates, as very acidic conditions can inhibit nitrogenase activities (Limmer and Drake 1996), while at close to neutral values, the N_2 fixation is known to be optimal (Roper and Smith 1991). This statement is further supported by our findings. Forest and grassland soils had pH below 7, and vineyard and arable around pH 7.9, explaining some of the observed

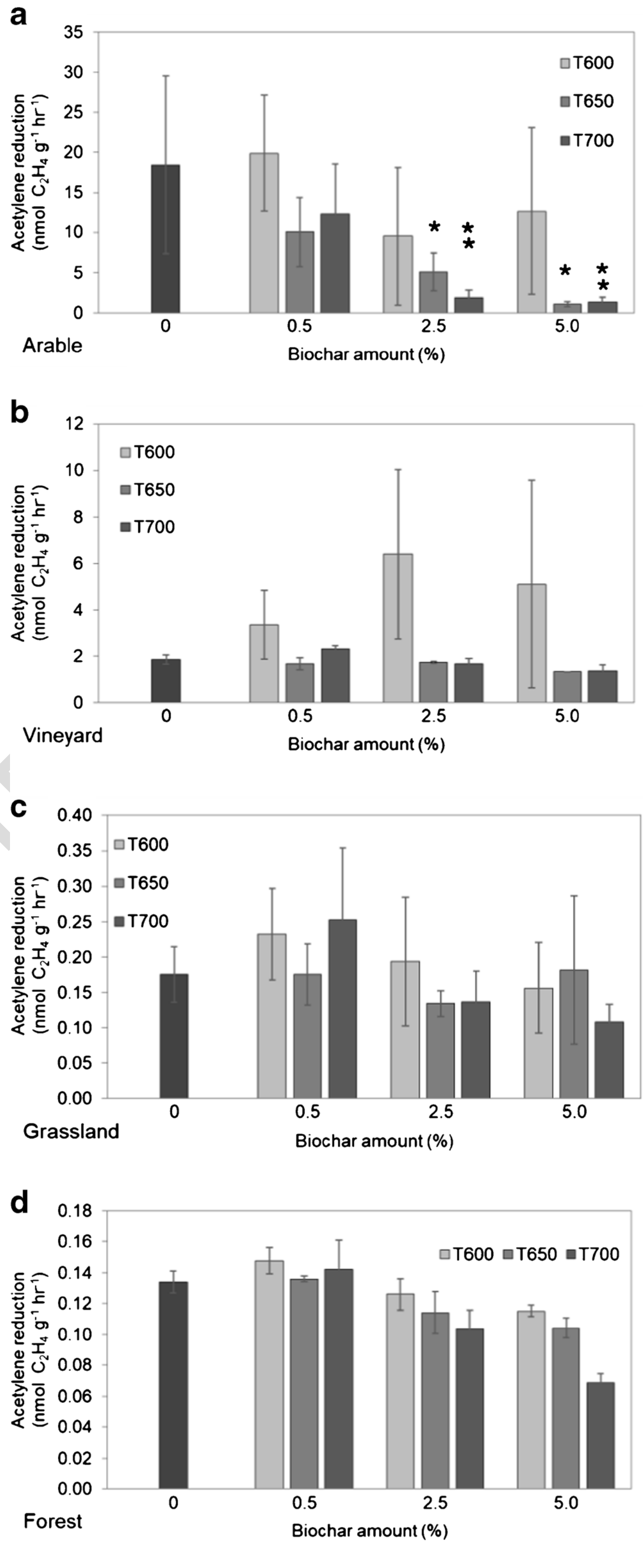


Fig. 5 Effects of the different biochar applications on acetylene reduction activity (ARA) values of **a** arable, **b** vineyard, **c** grassland, and **d** forest soils, based on C_2H_4 production rate. * and ** represent within land use type significance level of $p < 0.05$ and $p < 0.01$, respectively; $n = 3$, ± SD

ARA differences between land use types. Fertilizer addition to soils also has a major role in soil microbial activities. Cusack et al. (2009) found that fertilizer application can negatively influence BNF rates in tropical forest soils. Even though in the present study forest soils did not receive any fertilizer treatments, our investigated forest soils also showed some minor increases in ARA during spring and summer. Soil nutrient levels can also influence BNF rates. Mineral nitrogen can inhibit BNF, due to diversion of photosynthates to assimilate nitrates (Mulongoy 1995). The amount of nitrate present in soil can affect N_2 fixation. While a low amount of nitrate delays N_2O reduction to N_2 via BNF, a high amount of nitrates can suppress or even inhibit BNF process (Blackmer and Bremner 1978). In the present study, significant difference in NO_3^- measurements between seasons was observed only in the case of spring samples, while between land uses, the concentrations were relatively similar (Fig. 1b). When we investigated nitrate concentration and the BNF rates between seasons or land uses, no connections could be observed. Therefore, in our study, the amount of nitrate present in the soil was not a major controlling factor in BNF changes. Phosphorous deficiency in soil can decrease N_2 fixation along with causing reduction in nodulation and plant growth (Mulongoy 1995). Our findings further support this statement. Soil P_2O_5 concentrations below 50 mg kg^{-1} inhibited, while above 150 mg kg^{-1} enabled N_2 fixation (Fig. 4c).

Soil amendments such as activated carbon or biochar can also influence the availability of certain nutrients and soil moisture for plants and bacteria to use (Schiewer and Horel 2017; Thies and Rillig 2009). Biochars are generally lacking in inorganic nitrogen, which can enhance diazotrophs for colonization on the biochars' large surface area (Atkinson et al. 2010), consequently affecting the chemical and physical characteristics of the soil (Gaskin et al. 2010; Horel et al. 2018; Ouyang et al. 2013). With soil amendments, the oxygen levels can shift within the soil matrices, further influencing nitrogenase enzyme activities (Halbleib and Ludden 2000). Uzoma et al. (2011) investigated corn grain nutrient concentration changes as influenced by the different amounts of biochar addition to soils, and found inconsistent data, as total nitrogen decreased with low biochar addition and increased back to similar to control levels with higher biochar amount. Gaskin et al. (2010) observed only marginally increased yield in the case of corn when the effect of different plant-based biochars was investigated, finding additional amendments such as fertilizer to the soil necessary. Similar outcomes were perceived by Steiner et al. (2007), where the application of fertilizer and compost along with biochar resulted in substantial increase in yield, but biochar alone did not. Therefore, alongside biochar, fertilizer addition is also expected in agricultural lands to achieve better crop yield. This can influence soil BNF rates and overall plant growth and health on various scales depending on the type and amount of biochar, and the parameters and locations of the soil matrices receiving the supplements. Quilliam et al. (2013) investigated clover root nodules and N_2 fixation rates after 3 years of biochar application to temperate agricultural soils and found no influence of biochars as nodules had similar numbers and sizes, but the authors found increased nitrogenase activities. BNF rates are very high in legume plants, such as soybeans or peas (Masson-Boivin et al. 2009), and lower in non-symbiotic plant microbial connections with 3–306 kg N ha^{-1} $year^{-1}$ versus approximately 5 kg N ha^{-1} $year^{-1}$, respectively (Atkinson et al. 2010). In agricultural lands where crop rotation and/or fertilization occurs frequently, the symbiotic N_2-fixing plant microbial connections are more likely, helping atmospheric N_2-fixing processes further. In the present study, however, increases in ARA due to symbiotic BNF rates were unlikely to occur as no legume plants were present in any of the sampled sites. In our data, the differences in N_2-fixing potentials among the four land use types with or without biochar amendment signify the importance of free-living BNF in the nitrogen cycling processes. In our experiment, we used exactly the same amount of soil in all experimental setup while varying the biochar amounts; consequently, the originally present microbial amount could also be similar in all cases. When soils with high amount of biochar addition were investigated, the sudden decrease in N_2 fixation rates, especially in the case of arable soils, indicates potential negative effects of excess biochar application on N_2-fixing microbial activities.

In general, our results showed that in natural environmental conditions, acetylene reduction and consequently N_2 fixation rates vary significantly among different land use types and cultivation systems. These rates might be influenced by biochar application where microbial activities in non-legume-planted soils can be suppressed to the point that biological N_2 fixation might be reduced to a nominal level. These findings confirm the necessity for long-term studies investigating the effects of soil alterations on soil nutrient dynamics and nitrogen cycling processes.

Conclusions

Agriculturally more active areas such as tilled arable lands or vineyards can receive annual soil enhancers such as fertilizer or biochar additions, which can cause a disruption in its biochemical processes. Our study highlights that soil biological and chemical differences can be developed over time between land use types due to human interferences such as tillage, fertilizer addition, and crop rotation, as we found in the case of total N and SOC contents with higher values in the case of forest and grassland compared to arable or vineyard soils. Seasonal changes in environmental conditions can influence soil chemical changes, especially differences in nutrient concentrations in spring samples within land use types (e.g., NO_3^--N for all land uses or NH_4^+-N in the case of forest and grassland). Among land uses, we found that total N and SOC values can increase considerably during winter and spring for forest and grassland soils, mostly as a result of litter decomposition. In agreement with soil chemical changes, we also observed the influence of human impact on soil microbial communities, especially on ARA potentials. We found that the more interference occurred at a given land use site, the higher the potential BNF rates were (arable > vineyard > grassland > forest soils). The present study demonstrates that the smaller amount of biochar addition can increase, while the higher biochar amendments can inhibit BNF rates. However, the type of the biochar is also an important factor in the ARA rates, as we found the most positive impacts in biochar amendments prepared at the lowest pyrolysis temperature. Overall, our study emphasizes that careful planning and analyses should be implemented prior to soil enhancer additions to lands.

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