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Carbon quality affects the nitrogen partitioning between plants and soil microorganisms

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ABSTRACT

We investigated how the carbon quality of soil amendments based upon their carbon (C)-to-nitrogen (N) -ratio and their degree of aromaticity influence soil N transformations and affect N partitioning between soils, plants and microorganisms. A better understanding of these interactions might offer the possibility to optimize N use efficiency in agriculture. We performed a randomized pot experiment with winter wheat and compared the influence of naturally ¹³C labelled soil additives in three increasing condensation degrees, i.e. corn silage, hydrochar and pyrochar, in combination with three levels of ¹⁵N labelled NO3 on plant growth and N allocation. Corn silage, a lignocellulose material with a wide C-to-N-ratio and low condensation degree, which was also used as starting material for the two other amendments, favoured microbial growth and activity while simultaneously leading to N deficiency in wheat plants. In contrast, hydrochar and pyrochar positively influenced plant growth independent of their C-to-N-ratio and their degree of aromaticity. After adding hydrochar, plants did not take up the added fertilizer N but obviously used NH[‡] from mineralized hydrochar to meet their N demands. After adding pyrochar, fertilizer $NO_{\overline{3}}$ was used effectively by plants and fertilizer levels were still visible in the soil, while microbial activity was low. Our results clearly demonstrate that C quality strongly affects the N partitioning in the plant-soil-microorganism system. Hydrochars with a low degree of condensation that are slowly degraded by soil microorganisms might substitute N fertilizers whereas highly condensed pyrochars decreasing the soil microbial activity might enhance the N use efficiency of plants.

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1. Introduction

Agricultural use of soils is known for depleting their soil organic matter (SOM) content (Post and Kwon, 2000; Akça et al., 2005). This has adverse effects on plant growth and yield since SOM fulfils a plethora of important functions in soils, among others for soil aeration, water holding capacity and cation exchange capacity. Various measures have therefore been applied to minimize losses or to restore C contents by adding amendments like compost, biochar or other organic materials.

The various amendments added to soils in order to increase SOM contents vary in their C-to-N ratios and contain C of different quality: While uncharred organic matter provides mainly lignocellulose material to the soil, hydrochar adds C in the form of degree of condensation and pyrochar as higher condensed layers of interlinked aromates (Libra et al., 2011). All types of soil amendments do not only increase organic C contents, but they also interact with N transformations (Libra et al., 2011) according to their chemical and physical properties, especially, when mineral fertilizers are applied additionally. Biochars can alter the rate of N cycling in soils (Clough and Condron, 2010; Nelissen et al., 2012) and temporarily immobilise N in microbial biomass, especially if their C-to-N-ratio is wide (Gajic and Koch, 2012). Some studies found reduced N leaching rates in the presence of biochar (Ventura et al., 2013; Zheng et al., 2013), which might be caused by an adsorption of NO_3^- from the soil solution to the surface of the char material (Spokas et al., 2012) or a similar buffering of plantavailable NH₄⁺ (Nelissen et al., 2012). Different char materials may alter microbial nitrification and denitrification (Spokas et al., 2012). For example, Nelissen et al. (2012) found increases in gross mineralisation and nitrification rates, leading to an enhanced N turnover and a transfer of N from a stable pool into a more labile pool in the form of NH[‡]. The addition of C to soil via organic amendments might favour the process of dissimilatory NO_3^- reduction to NH_4^+

aromatic structures arranged as spherical bodies that have a low







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(DNRA) especially under slightly anaerobic conditions and in the presence of a wide C to NO_3^- ratio (Rütting et al., 2011). On the other hand, N₂O emissions can decrease in the presence of char materials (Malghani et al., 2013; Zheng et al., 2013) which might favour the last step in denitrification, thus decreasing the ratio of N₂O/N₂+N₂O in soil N emissions (Cayuela et al., 2013). This effect, however, seems to be dependent on the nature of the added soil amendment, since other authors found increases in N₂O emissions in the presence of hydrochar char while pyrochar decreased these emissions (Kammann et al., 2012).

Despite the available information, little is known about the effects that differences in C quality exert on the interaction between C-containing soil amendments and the N allocation between plants and soil organisms. A better understanding of the related interactions might offer the possibility to minimize the agricultural impact on ecosystems by using the gained knowledge to optimize agricultural management (Spokas et al., 2012). Clough and Condron (2010) thus recommend research on the effects of various biochars on N transformations and especially on the fate of N additions to soils amended with char materials. The comparison of different materials offers insights into the interaction of C and N cycling in agricultural ecosystems and help to improve N use efficiency of agricultural crops. We expect that the differences in organic matter quality cause related variations in the allocation of N. We hypothesize (1) that soil amendments with a high C-to-N-ratio and a low condensation degree immobilise N thus causing N deficiency in plants if no N fertilizer is added to the system and (2) that soil microbes are progressively inhibited in the presence of materials with increasing structural organization of aromatic compounds. To test these hypotheses, we set up a pot experiment with winter wheat growing in soils amended with different organic materials all based on corn silage at various levels of ¹⁵N-labelled fertilizer.

2. Material and methods

2.1. Experimental design

A greenhouse experiment was conducted to compare the effects of organic amendments differing in their C-to-N ratios or their physico-chemical structure - corn silage, hydrochar (produced from corn silage by Fa. CS Carbon Solutions, Kleinmachnow, Germany) and pyrochar (produced by the authors from corn silage) - on plant growth and soil properties. Chemical characteristics of the material are described by Malghani et al. (2013). 54 pots with a diameter of 10 cm were filled with sandy soil, 54 pots were filled with calcareous soil (Table S1). Half of the pots of both soil types were planted with nine seeds of winter wheat (Triticum aestivum L., type "Bussard", Bioland Jeebel, Germany) each; the other half was left without vegetation. The amount of added amendment increased the organic C content of the original soil by 30% and corresponded to 3.6, 3.5 and 2.0 weight% for silage, hydrochar and pyrochar, respectively. All three types of additives were produced from corn silage, thus adding a δ^{13} C label to the soils (Table 1). Three different levels of N fertilizer were applied after germination: no fertilizer, 10 mg $KNO_3 - N$ per pot and 20 mg KNO_{3} – N per pot, corresponding to a fertilizer application of 0,

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12.5 and 25 kg N ha⁻¹. KNO₃⁻¹ containing ¹⁵N (10 atom%) was added in order to be able to track the partitioning of N between plants, microorganisms and soils. The total number of independent replicates per treatment was three.

All pots were distributed randomly on a table in the greenhouse and watered regularly three times per week. The appropriate watering amount was defined by previously performed watering tests with untreated soil to investigate the water holding capacity and the adsorption quantity of the different soils. Some representative pots were weighed to estimate the water loss and pots were refilled accordingly. During the course of the experiment, pots with plants progressively needed more water and, consequently, the watering amount was adjusted to keep the soil moisture constant. This included sometimes postponed watering in soils treated with pyrochar, as their soil moisture persisted at a high level for a longer period of time after watering.

2.2. Measurements

2.2.1. Plants

All pots containing seeds were checked for seedlings on days 1, 2, 3, 4, and 7 after sowing. Height measurements of all emerged plants were performed on days 9, 11, 15, 17, 21, 28, 31 and 38/39 after sowing in order to check for differences in growth rates between the treatments. On day 38 after sowing, chlorophyll fluorescence was measured on three representative leaves per pot with a portable chlorophyll fluorometer PAM-2000 (Walz Mess- und Regeltechnik, Effeltrich, Germany) connected to a HP 200LX Palmtop PC (Hewlett Packard GmbH, Böblingen, Germany) in order to get an objective measure for plant vitality. Leaves were shaded with leaf clips 10 min prior to the measurement in order to get a value for minimal fluorescence in the dark (F_0). Via the application of the pulse amplitude modulation technique, maximum emission of fluorescence during a saturating light pulse (F_m) and variable fluorescence ($F_v = F_m - F_0$) were determined. The ratio F_v/F_m is an indicator for the performance of photosystem II and is lowered when plants experience stress (Krause and Weis, 1991; Maxwell and Johnson, 2000; Baker, 2008; Murchie and Lawson, 2013). It can thus serve as an indicator for plant vitality.

Aboveground plant parts were harvested on day 39 after sowing. Leaf area of three leaves per pot was measured with a leaf areameter LAI 3000 (Licor inc., Lincoln, Nebraska). Dry weight was determined separately for the measured leaves and the rest of the biomass per pot after drying the samples at 70 °C for 5 days. Roots were carefully removed from the soil, washed and dried at 70 °C to determine their dry weight per pot.

The dried plant samples were milled and subsamples were analysed for organic C and N with a Vario Max and a Vario EL (Elementar Analysensysteme GmbH, Hanau, Germany), respectively. Further subsamples were analysed for δ^{15} N values with a Delta+ (Thermo Finnigan MAT, Bremen, Germany) coupled online to an elemental analyser EA 1100 (CE Instruments, Milano, Italy) via a ConFlo III (Werner et al., 1999; Steinbeiss et al., 2008a; Gubsch et al., 2011).

	C [%]	N [%]	C/N	Sand [%]	Silt [%]	Clay [%]	pH 0.01 M CaCl ₂	δ ¹³ C [‰]	δ ¹⁵ N [‰]
Calcareous soil	1.8	0.20	18	9.2	75.1	15.7	7.5 (1:2.5)	-26.37	6.81
Sandy soil	5.3	0.29	9	50.4	43.8	5.9	6.8 (1:2.5)	-27.82	5.94
Corn silage	43.7	1.22	36	_	_	_	4.6 (1:8)	-12.33	7.05
Hydrochar	40.6	2.45	17	-	_	_	5.4 (1:1)	-12.80	6.88
Pyrochar	73.7	2.20	34	-	_	_	10.6 (1:8)	-12.75	7.46

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