SOIL MINERAL NITROGEN TRANSFORMATION IN TERMS OF BIOCHAR AMENDMENT ALONG WITH MINERAL ADDITIVES AND INOCULUMS INFLUENCE

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Abstract: The carbon rich solid formed by pyrolysis of biomass or "biochar" with its storage in soils considered to be as a mean of climate changes mitigation by sequestering carbon. Investigators argue biochar's effectiveness as a global warming solution due to its remaining stable in the soil for many years and its positive effect on soil fertility with special its particular chemical and biological properties. However, soil treatment with a freshly prepared biochar may cause a danger of plant growth deterioration. The main target of the following research is to investigate the effect of biochar amendment along with the microbial inoculums and fertilizers addition on soil mineral nitrogen transformation by measuring its content in test soil and the availability of nitrogen for soil microbes. Different variants with biochar treatment along with two types of inoculums and mineral fertilizer amendment have been adjusted in a growth box during the experiment conduction. Lettuce (*Lactuca sativa*) is considering as a sensitive indicator to soil state changes hence it has been chosen as an experimental test-plant. Determination of nitrogen availability and measurement of mineral nitrogen leaching have been analyzed. It has been observed that the application of inoculums along with biochar amendment supports microbial activity and consequent nitrogen immobilization that has been accompanied with low values of bothNH_4^+-N and NO_3^--N losses.

Key Words: biochar, inoculum, *Lactuca sativa*, mineral nitrogen, soil

INTRODUCTION

Environmental problems in Eastern Europe illustrate the years of neglecting environmental regulations that have led to deteriorated soil quality with insufficient soil organic matter amount, soil acidification, salinization and overheating. Hence finding new appropriate possibilities and technologies for soil revitalization or remediation becomes of a great urgency at the present days. Nowadays biochar amendment into soil is one of the most increasingly discussed controversial and powerful tools to combat climate change and increase soil fertility by sequestering atmospheric carbon (Hunt et al. 2010, Zimmerman et al. 2011). Great amount of authors state that biochar has also been shown to change soil biological community composition along with microbiological variety and abundance. Moreover, this amendment may influence nutrient cycles, organo-chemical, physical properties of the soil and has been shown to increase soil microbial biomass (Jin 2010, Lehmann et al. 2011, Liang et al. 2010, Czimczik et al. 2002, Nguyen et al. 2010). In addition, some researches claim that the greater mineralizable fraction of biochar is the greater N immobilization occurs. As a result decreases nitrogen uptake and crops growth (Deenik et al. 2010). Furthermore, larger microbial biomass fixed with biochar additions will certainly contribute to both effects. Moreover, biochar application transforms soil nitrogen dynamics (Clough et al. 2013). Some authors describe biochar derived from pecan shells for example to reduce nitrate leaching from soil over 25 and 67 days (Novak et al. 2009). Biochar has been shown by the researches to have perspective in reducing inorganic-N leaching, N_2O emissions, ammonia volatilization and biological nitrogen fixation increase. The reduction of N leaching may be tightly connected with NH_3 adsorption or organic-N onto biochar, cation or anion exchange reactions and enhanced N immobilization consequently of labile C addition in the biochar (Singh 2010, Spokas 2009, Steiner 2010, Rondon 2007). Researchers have found that less NO_3^- leaches when the lowest temperature biochar is presented which is mainly explained by the presence of easier degradable C compounds (at the lowest temperature) and greater N immobilization that results inNO_3^- leaching reduce (Ippolito et al. 2012).The cation exchange capacity (CEC) of biochar is the reason of the NH_4^+ adsorption onto biochar and the observed reductions in NH_4^+ leaching. Some authors have concluded that biochar adding to the soil potentially increases microbial nitrogen cycling, particularly the abundance of those organisms that may decrease N_2O fluxes and NH_4^+ concentrations (Anderson et al. 2011).

The key goal of our research is to conduct an investigation on nitrogen availability for soil microorganisms and mineral nitrogen leasing estimation in terms of biochar application into the soil along with inoculums and mineral fertilizer addition.

MATERIAL AND METHODS

Characterization of experimental design

Research experiment has been conducted in laboratory conditions during the period from December 2015 until April 2015 in a special growth box phytotron with the next constant ambient conditions: 24°C daily temperature, 20°C night temperature, 65% humidity with a day length of 12 h and light intensity of 380 $\mu mol \cdot m^{-1} \cdot s^{-1}$. *Lactuca sativa* has been chosen as an indicator test plant. In the course of our research twenty plastic experimental containers have been used that have been subsequently filled with 1 kg of topsoil from the protection zone of underground water drinking source "Březová nad Svitavou" (49°38'39.2"N 16°31'04.3"E). Soil samplings have been conducted according to ČSN ISO 10 381-6 (ČSN - Czech Technical Standard). After soil samples have been homogenized and sieved through a sieve with a grid size of 10 mm. In addition, rhizosphere and non-rhizosphere root zones have been separated with special UHELON 130 T uni mesh bags.

Beech wood biochar have been applied during the investigation into experimental containers with test soil. This particular type of biochar has been made in low temperature sat about 350°C – 500°C with the slow pyrolysis application. Further to the biochar amendment specific inoculums "Bactofil" and "NovaFerm" have been added. Five experimental variants in four repetitions have been prepared to analyze biochar amendment effect along with inoculums addition (see Table 1).

Table 1 Overview of applied treatments

Variants	Amendment	Application rate	BBCH	Active ingredients
V1 (Control)	-	-	-	-
V2	Biochar dose +"Bactofil" inoculum	50 t ha^{-1} 1 l · ha^{-1}	13 15–18	*Azospirillum brasilense, Azotobacter vinelandii, Bacillus megaterium, Bacillus polymyxa, Pseudomonas fluorescens, Streptomyces albus*
V3	Biochar +"Bactofil" inoculum+ DAM 390	50 t ha^{-1} 1 l · ha^{-1} 140 kg N ha^{-1}	13 15–18	*Azospirillum brasilense, Azotobacter vinelandii, Bacillus megaterium, Bacillus polymyxa, Pseudomonas fluorescens, Streptomyces albus, mineral nitrogen*
V4	Biochar +"NovaFerm" inoculum	50 t ha^{-1} 10 l · ha^{-1}	13 15–18	*Azospirillum spp., Azotobacter spp., Bacillus megaterium, Bacillus subtilis*
V5	Biochar +"NovaFerm" inoculum+ DAM 390	50 t ha^{-1} 10 l · ha^{-1} 140 kg N ha^{-1}	13 15–18	*Azospirillum spp., Azotobacter spp., Bacillus megaterium, Bacillus subtilis, mineral nitrogen*

Determination of nitrogen availability

Determination of nitrogen availability index is based on the method of available nitrogen content measuring in soil. The procedure has been established and described by the authors (Bundy, Meisinger 1994). This particular approach is divided into two experimental stages. The first stage is used to determine mineral nitrogen content before soil incubation. The second stage is based on evaluation of ammoniacal nitrogen content that is fixed after soil incubation within 7 days with the 4 M potassium chloride application. Consequently NH_4^+ is released mainly from microbial biomass cytoplasm where it has been generated. Available soil nitrogen is estimated from NH_4^+-N.

Measurement of mineral nitrogen leaching

Measurement of mineral nitrogen leaching (N_{min}) has been estimated according to the authors (Novosadova et al. 2011). N_{min} loss evaluation has been conducted using Ion Exchange Resins (IER) which have been placed into plastic PVC discs situated under each experimental container (see Figure 1). These discs have been made from plastic (PVC) tubes. Each disc is of 75 mm diameter and is 5 mm thick. Nylon mesh with a grid size of 0.1 mm has been glued from the both sides of each disc. Mixed IER (CER – Cation Exchange Resin and AER – Anion Exchange Resin in ratio 1:1) have been placed into the inner space of an annular flat cover. In the end of the experiment these discs have been dried at laboratory temperature 18.5°C for seven days after an exposition under the experimental containers. N_{min} has been extracted from individual discs with IER using 100 ml of 1.7 M sodium chloride. Distillation-titration method has been performed for the determination of released N_{min} according to (Peoples et al. 1989). Obtained results have been expressed in mg of N_{min}.

Figure 1 Lactuca sativa in the terms of laboratory conditions

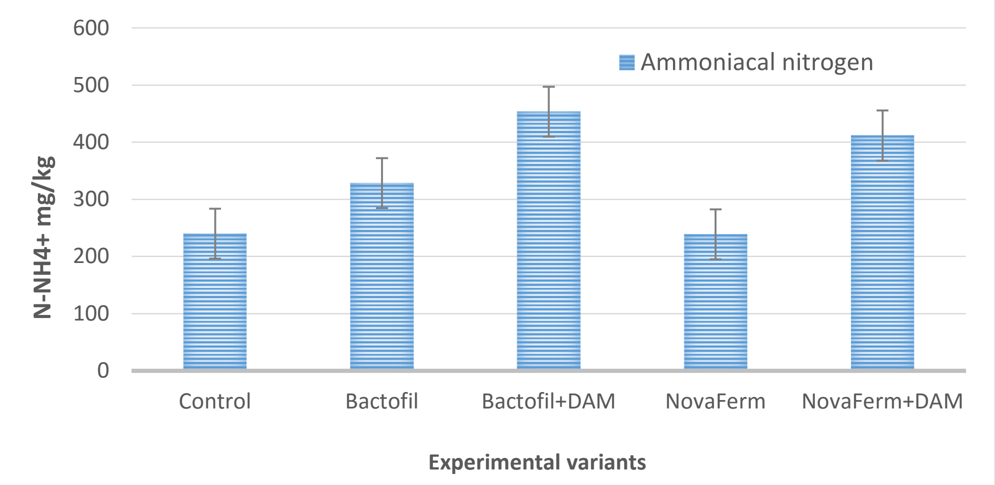
RESULTS AND DISCUSSION

Availability of nitrogen for soil microbes

Ammonium nitrogen evaluation indicates the NH_4^+-N amount in the microbial biomass. This compound has been estimated in filtered extracts after waterlogged incubation within 7 days. Nitrogen availability for soil microbes in arable soil has been measured according to the authors (Bundy, Meisinger 1994). Nitrogen availability index (that is of ammonium production) during waterlogged incubation is applied usually to estimate nitrogen amount stored in the microbial biomass.

Experimental results state on the following trends (see Figure2). It has been set that the highest N availability has been found in the V3 variant (453.27±16 mg.kg^{-1}) with the application of both additives and mineral fertilizer – biochar, „Bactofil“ inoculum and DAM fertilizer compared to the control sample V1 (239.86±12.4 mg.kg^{-1}). Researchers has found out that microbial inoculums with a broader range of species may have a positive effect generally since it is more likely that at least one or more organisms will survive or even thrive under adverse soil environments. In addition, assimilation of nutrients within microbial biomass enhances the retention and recycling of these nutrients. Furthermore, after organisms decomposition they subsequently become an energy source for other organisms within the soil food web (Kalogridis et al. 2006). It may be stated that carbon mineralization is greater than it has been expected as positive priming for soils mixed with biochar produced at low temperatures (250°C and 400°C) has been occurred (Zimmerman et al. 2011). However, experimental results stated on N availability decrease in the variants with DAM mineral fertilizer amendment along with investigated inoculums.

Figure 2 NH_4^+-N availability in microbial biomass (mean values ± standard error; n=4)

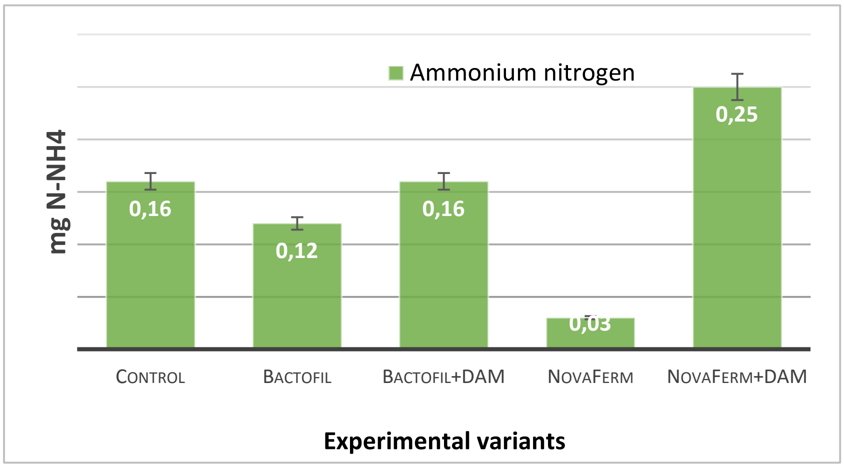


The V5 variant showed N availability high rates as well (411.68±19 $mg.kg^{-1}$) with the biochar along with „NovaFerm“ inoculum and DAM amendments. Not of such a great significance but still positive effect is observed in V2 variant (328.69±11.5 $mg.kg^{-1}$) where „Bactofil“ inoculum effect prevails. The lowest nitrogen availability index has been fixed in V4 (238.68±13 $mg.kg^{-1}$) variant with „NovaFerm“ inoculum addition influence that is almost equal to the control sample. This latter data argue on reduced NH_4^+-N availability in microbial biomass that may be explained by the fact that inoculum only addition with already presented biochar in soil has insufficient amount of organic nutrients that are essential as an energy and a supply source for soil microorganisms. Subsequently soil microbiota fail to use soil nitrogen and after store this compound in their bodies.

Mineral nitrogen content in soil

Mineral nitrogen content have been estimated in the experimental soil with two basic values ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) which are considered to be important indicators of soil negative impacts caused by nitrogen saturation. Biochar amendment effect along with inoculums and mineral fertilizer addition on ammonium nitrogen NH_4^+-N content in arable soil illustrates Figure 3.

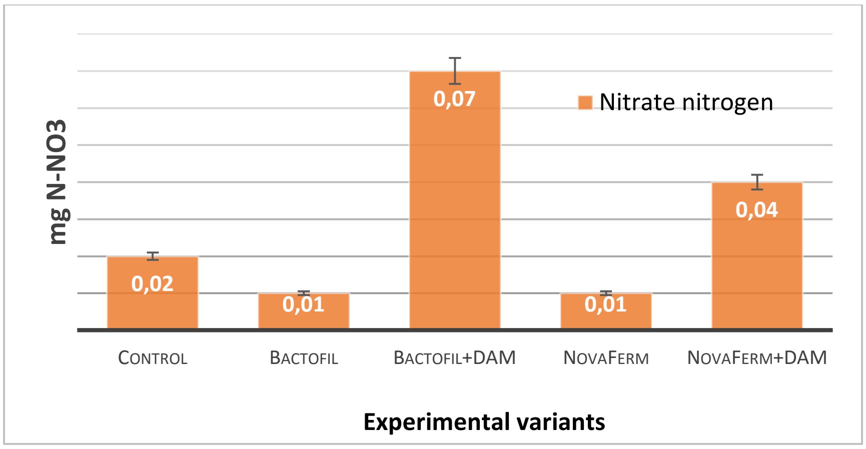
Figure 3 NH_4^+-N leaching from arable soil (mean values ± standard error; n=4)



Presented data state on the significant NH_4^+-N leaching decrease in combined variant V4 with biochar and "NovaFerm" inoculum additives (0.03±0.01 mg) comparing to the control sample V1 (0.16±0.03 mg). The highest NH_4^+-N leaching has been observed in V5 (0.25±0.03 mg) variant with the inoculums treatment and mineral nitrogen fertilizer DAM amendment. Insignificant but still positive effect and NH_4^+-N leaching reduction has been obtained in V2 variant with "Bactofil" application (0.12±0.02 mg). Hence, application of inoculums together with biochar amendment and without mineral fertilizer addition supports microbial activity and nitrogen immobilization that has been proved by the lowest values of NH_4^+-N loss in the studied variants.

Research results of NO_3^--N loss from experimental soil indicate almost identical rise and fall of investigated values in comparison with the results of NH_4^+-N leaching (see Figure4).

Figure 4 NO_3^--N leaching from arable soil (mean values ± standard error; n=4)



In the studied variants V2 and V4 with only inoculums treatment low decrease trend has been recognized (0.01±0.005 mg and 0.01±0.003 mg respectively) compared to the control variant (0.02±0.007 mg). Visible NO_3^--N leaching increase has been obtained in the variants V3 and V5 with the DAM mineral fertilizer amendment in contradiction to the dramatic drop and beneficial effect of inoculums addition in V2 and V4 variants. This tendency may approve a hypothesis that argues on nitrogen fertilizers addition that leads to an increase of the utilizable nutrients contents.

CONCLUSION

Final investigation results demonstrate that biochar treatment can affect soil properties in various ways dependent on the addition and application of particular inoculums and mineral fertilizers. Research data of the determination of nitrogen availability for soil microorganisms show high NH_4^+-N availability in terms of biochar treatment along with inoculums adding and mineral N fertilizers that may support the increase of soil mineralization. From the other hand, application of only inoculums along with biochar amendment and without mineral fertilizers treatment supports microbial activity and nitrogen immobilization that has been proved by lower values of NH_4^+-N loss. Moreover, the same positive effect with biochar treatment and inoculums amendment into the soil has been obtained in the case of NO_3^--N loss investigation from the experimental soil.

Further experimental research is planned to be targeted on applying and planting into the same studied soil but with already changed physical, chemical and biological properties the second generation of test plant lettuce (*Lactuca sativa)*in order to study biochar's properties with mitigated effect on investigated plant.

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