Chemically and biologically-mediated fertilizing value of manure-derived biochar

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Abstract: This study evaluates the potential of manure-derived biochars in promoting plant growth and enhancing soil chemical and biological properties during a 150 day pot experiment. Biochars from pyrolysis of poultry litter (PL) and swine manure (SM) at 400 and 600°C, and a commonly available wood chip (WC) biochar produced at high temperature (1000 °C) were incorporated to silt-loam (SL) and sandy (SY) soils on a 2% dry soil weight basis. Ryegrass was sown and moisture was adjusted to 60% water filled pore space (WFPS). The PL400 and SM400 biochars significantly increased ($p<0.05$) shoot dry matter (DM) yields (SL soil) and enhanced nitrogen (N), phosphorus (P) and potassium (K) uptake by the plants in both soils, compared to the Control. All biochars significantly increased the soil carbon (C) contents compared to the Control. Total N contents were significantly greater for PL400 and PL600 treatments in both soils. The dehydrogenase activity (DA) significantly increased for PL400 and SM400 treatments and was positively correlated with the volatile matter (VM) contents of the biochars, while β-glucosidase activity (GA) decreased for the same treatments in both soils. All biochars significantly shifted ($p\leq0.05$) the bacterial community structure compared to the Control. This study suggests that pyrolysis of animal manures can produce a biochar that acts as both soil amendment and an organic fertilizer as proven by increased NPK uptake, positive liming effect and high soil nutrient availability, while WC biochar could work only in combination with fertilizers (organic as well as mineral).

Response to Reviewers: Dear Editor,

Please see the docx file attached together with the revised version of the manuscript for the detailed response to Reviewers' comments. We fully agree agree with most of the comments raised by the reviewers and have substantially revised our manuscript accordingly (attached cover letter). Please note that the blue coloured texts in the revised version of the manuscript represent the one with changes clearly marked (attached in the zip file). In addition, one copy of same revised version with no changes

marked (plain texts) has also been attached together for publication-ready.

Thank you for accepting our submission of the revised version of manuscript into STOTEN.

Regards,
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Prof. D. Barceló Cullerés, Prof. J.P. Bennett
Co-editors-in-chief
Science of the Total Environment

Subject: submission of revised manuscript for publication

Dear editor:

I am sending you the final version of the manuscript entitled, **"Chemically and biologically-mediated fertilizing value of manure-derived biochars"** after a new revision. Please note that, following a proper suggestion of Reviewer #1, we have slightly re-formulated the hypothesis no. 3 of our study in order to well relate the obtained results back to experimental hypothesis.

The manuscript has been substantially modified following the constructive comments and suggestions of two anonymous reviewers and the Associate Editor, as described in the Revision Notes. We fully agree with almost all the points raised by both reviewers, while we have put our arguments in relation to those comments we did not agree.

The primary objective of our study is solely to evaluate the agronomic value/potential of manure biochars, and investigate whether pyrolysis of manure could be a potential option in livestock manure processing, as a mean to stabilize organic matter in the feedstock, and recover nutrients in the char. In such case, technical and economic feasibility of manure pyrolysis would require a separate case study to have complete picture on manure processing that includes life cycle analysis of the entire system.

We believe our study further advances the knowledge in understanding the limits and the potentials connected to the use of manure/waste-derived biochars as Europe works towards attaining the mitigation strategies applicable to manure/waste management.

We would be grateful if you would reconsider the manuscript for inclusion in a forthcoming issue of your journal. Thank you very much.

Yours faithfully,

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Response to reviewers' comments

Reviewer #1: General Comment

Comment 1.1: The manuscript reports the effects of two manure-based biochar materials produced at 2 temperatures and a wood biochar on rye grass growth two soils with different texture. The topic matches the aim and scope of the journal, and presents an interesting dataset demonstrating differential impacts of biochars on plant growth and plant nutrient dynamics. The manuscript can be improved by 1) relating experimental results back to hypotheses, this may help the authors better address observed differences in the two soil types; 2) improving the integration of the soil biological measurements within the context of the experiment and the often cited Subedi et al (In Press); 3) there needs to be more thought regarding the inclusion of the DGGE data, as it stands these do not inform or add much to the manuscript. Overall, I enjoyed reading the manuscript.

Authors: We learned that some of the points raised by the reviewers in our manuscript were not clear. We are grateful to both reviewers for their keen interest in our study results. Based on the constructive comments and suggestions received from them, we have substantially revised our manuscript.

Please kindly note that following the suggestion from reviewer #1, we have slightly re-formulated the hypothesis no. 3 of our study in order to well relate our results of soil chemical and microbial properties back to the experimental hypothesis.

Specific Comments

Comment 1.2: P4L48-55: It seems that "..main drivers.." is too strong a statement. How can the authors are claim that biochar driven effects on nitrification etc are the main drivers for increased plant growth? I am not sure there is enough evidence for such a broad claim. This may be the case in some isolated studies, but not broadly speaking.

Authors: New page 4: We agree with the reviewer. Our wording is now changed.

Comment 1.3: P6L21-26: First, it would be helpful to have some taxonomic information on the soils above just their textural class. What is the mineralogy of the soils, for example? Also, what does sub-acidic mean? These appear to be slightly acidic soils (pH=6.1) and alkaline soils (pH 8.3), at least from a general soil science perspective.

Authors: New page 7: We agree with the reviewer on the definition of the soil pH class. The text is changed accordingly (slightly acidic, moderately alkaline). We have also added an information on soil taxonomy, being the two soils an Alfisol and an Entisol (USDA, 1999) (Section 2.1).

Comment 1.4: P7L21: I could find no information on extraction procedure for Heavy metals nor analytical procedures. Details for metals analysis are not in Subedi et al. (in press).

Authors: New page 8: This is true for Heavy metals, but also for other analysis. In fact another reviewer is also asking for more details on methods on all results of table 1 and 2. In the revised text we have now included an explicit reference for each analysis. On top of that we still give a little more details on Heavy Metals.

Comment 1.5: P16L26: What is remarkable about the results, especially given the fact that these DM responses to biochar fall within the expected positive responses reported in the literature. It would be appropriate to state right away that the positive effects on DM were really limited to the finer soil.

Authors: New page 18: We agree with the reviewer and the new sentence is limiting our positive remarks only to some specific type of biochar and making a distinction between soil types (page 18: 3rd paragraph).

Comment 1.6: P16L43-58: What is the point of referring to other literature showing that not all crops respond equally to the same biochar application? What is the connection to the present research?
Authors: New page 18: We think that our comment is important and it is in the line of the reviewer previous comment (P16L26). It shows that our experimental results cannot be considered "fully remarkable" because we used only one single crop. This is the reason for which we would prefer to leave the sentence as it is (page 18: last paragraph).

Comment 1.7: P17L11-19: Perhaps there is indirect evidence for increased N mineralization due to higher N uptake in the biochar amended soils, but where is the support for "increased nutrient retention on exchange complex" especially given that authors report no biochar effect on CEC in Table 4?
Authors: New page 19: We agree with the reviewer because it is true that CEC did not increase and also because our pots did not really expose N to leaching and N retention probably played a secondary role. Therefore we removed the second half of the sentence.

Comment 1.8: P17L24-26: There is no data or evidence provided in this investigation that links biogeochemical S to improved rye grass DM.
Authors: New page 19: We agree that there is "not data or evidence", but here we are trying to report the possible reasons behind a higher measured DM yield. In fact we write "may be due to". We would like to leave this sentence in our discussion to show (also basing on the literature) that the explanation of such effects could be larger than considering only N and P. In addition, Subedi et al. (2016) has reported significant water soluble sulphates on manure biochar treated soils which can be linked to S mobilization by the bacterial, potentially changing biogeochemical S cycle.

Comment 1.9: P18L9-13: The lack of a strong link between NPK uptake and DM yield in the SY soil is puzzling, but how can the authors suggest that biochar was effective in the SL because it was acidic and not in the SY because it was not acidic. How can a soil with a pH of 6.56 be considered acidic? The authors have not provided an adequate explanation for the general lack of DM response to biochar in the sandy alkaline soil.
Authors: New page 20: Following the reviewer comment, we observed that the increase in NPK uptake compared to Control (mainly for PL400 and SM400) in SY soil was not by DM increase, but due to increase in tissue NPK concentrations in DM (despite the overall NPK uptake still lower in SY soil than in SL), while opposite was true in SL soil. So, we noted that the general lack of DM response in SY soil could be due to low CEC, low organic matter and strong soil alkalinity (pH between 8.5–9.5). We hope this clarifies.

Comment 1.10: P18L28: What do the authors mean by "indirectly available" P?
P18L33: PL00?
Authors: New page 20: Corrected, it is PL400. We would regard the majority of P to be not directly plant available as P is bound organically or inorganically. In particular the organically bound P can be cleaved enzymatically by many soil microbes including fungi and bacteria. This release of organically bound P will then become available to the plant that is unavailable in the absence of the soil microbes.

Comment 1.11: P19L9: Please provide support for the statement "..expected to be higher…" in relation to biochar N mineralization.
Authors: New page 21: We are supporting this statement linking the expected higher N mineralization with the lower C/N ratios of low temperature manure biochars compared to high temperature biochars. We added this in the revised text (page 21: 1st paragraph).

Comment 1.12: P19L26-35: High in relation to what? In relation to regulatory standards? This is an important point that must be better addressed. Also, the sentence beginning "As we did not…" is convoluted and difficult to decipher. Importantly, not measuring metal accumulation in the plant shoots is

a weakness in the study, especially if biochar metal content is considered "high." The authors should take the time to address bioavailability of metals in their soils.
Authors: New page 21: Following this observation we performed further literature investigation. We found that the measured values for heavy metals content are below the new official threshold limits set by the European Biochar Certificate and also International Biochar Initiative. We have included this information in the revised text. This justifies the lack of attention to this aspect in our experiment which is a very short duration experiment not suitable to validate potential accumulation of heavy metals in the soil after several years of poultry biochar amendment (page 21: 1st paragraph).

Comment 1.13: P19L43: What is meant by "…direct transformation of these elements into soil-biochar matrix?" This sounds like jargon. Especially since this sentence is followed by a discussion of SOC with no reference to "these elements."
Authors: New page 21: We agree with the reviewer that our sentence was unclear. Here the nutrients mean both macro & micro nutrients, and this sentence also covers the all following paragraphs about nutrient (N, P, K, Ca and Mg) increase with biochar additions. The new sentence is: The increase in nutrient contents of biochar-amended soils is mainly associated with the 'direct addition' of these elements (page 21: 2nd paragraph).

Comment 1.14: P20L53-58: Explanations for K availability are not well articulated. What does this mean: "Again, the explanation for this could be similar with that of P contents in the SL soil even though there is no clear evidence for this."
Authors: New page 23: We mean to say that the average exchangeable K content in soil, over all treatments, in SL soil is lower (1.6 times) compared to that of SY soil, simply because K uptake in SL soil was much higher than in the SY soil. This articulates that plant was able to deplete significant K in SL soil (because it had high DM yield) than in the SY soil. We have elaborated this aspect in the main text (New page 23). We further explored that Ca may have competed more with K for the exchange sites in SL soil due its higher affinity as the Ca availability is higher in SL soil than in SY. It is also true that a near neutral pH normally reduces the amount of K in the soil solution, as the SL soil used in this study had pH range of 6.5–7.8 (Sachs, 2004). We hope our new explanation is valid for K.

Comment 1.15: P21L19-21: For the explanation of low Ca due to biochar, is the binding with P the only explanation for lower Ca in biochar amended soil? If this is the explanation, how do the authors explain increased P availability with biochar addition if it is binding with P from biochar. Please help clarify this. How can low P uptake in the SY soil "corroborate this" when Olsen P increased nearly 10-fold with biochar compared with the Control? How did biochar addition affect Ca uptake?
Authors: New page 23: We noted that the total P supplied from the manure biochar (Table 2) to each soil is significant (up to 575 mg P from PL600 biochar), and only 44-61% of total biochar P is available, while portion of these available fraction as well as unavailable part might have been fixed with either Ca and/or other elements (Mg), leading to the formation of insoluble Ca-phosphate. In addition, as the SY soil used in this experiment was highly calcareous (15% CaCO3), significant amount of Ca is unexchangeable as this could have been precipitated as insoluble $CaCO_3$.
We further understood that the low exchangeable Ca in SY soil is also regulated by the soil pH as the Ca availability starts decreasing slowly pH above 8.5. In addition, the less available Ca in SY soil may be due to the formation of Ca-oxalate, a byproduct of fungal weathering (e.g. Schmalenberger et al Sci. Report 2015), which could be increased around the biochar particles.
Finally, low P uptake in SY soil is independent to P availability in this soil, but due to the low DM yield. The low DM yield in such poor SY soil is mainly linked to the restricted plant growth as it has lower CEC and OM compared to the SL soil, also due to strong soil alkalinity. We hope this is clear now.

Comment 1.16: P21L28-54: It would be helpful to begin discussion of MBC in reference to the appropriate hypothesis. This paragraph in its present form is difficult to follow and doesn't add anything

particularly useful to the research. MBC doesn't seem to be correlated to anything, and its very different behavior in the two soils is not explained. Also, MBC did not remain stable in the presence of biochar. What is the significance of "…a shift in microbial community structure." These are descriptive data with no functional meaning.

Authors: New page 24: We agree with the reviewer that our result on MBC is completely different in two soils and is not correlating with any other soil properties. Thus, we have completely restructured and slightly expanded this part by providing a more presentable idea with evidences after going through more literatures. However we admit that this part of our work "begs further experimental verifications" (as we write in our new page 24: last sentence)

Comment 1.17: P24L26-58: Difficult to see the importance and/or significance of including the DGGE data. As presented in the manuscript, these are strictly descriptive data that add no interpretive value to the research. To add meaningfully to the field, the authors need to enhance these data with some functionality.

Authors: New page 27: We have now better integrated the community profiling data into the discussion as recommended and have made further interpretations. While this form of analysis does not allow for direct functionality analysis (this would be an entire new study), the presented data reveal insights into general bacterial diversities and structures that are based on a universal marker (16S rRNA gene). Interesting to note here is the fact that the analyzed bacterial communities are significantly affected by all applied biochars. Furthermore, PL400 could be singled out by the DGGE analysis in SY, thus at least for SY, a plant growth promotion effect is significantly correlated with a change in bacterial community structure. Through discussion with other recent publications, we concluded our findings suggest that soil bacteria contribute to the observed plant growth promotion effect alongside the effect of biochar on the physico-chemical status of the soils. We have now made this line of thought clearer in the discussion and hope that this is now adequate to warrant a publication.

Reviewer #2: General Comment

Comment 2.1: The paper is well written; the experimental set-up is thoroughly planned, executed and described, the results are well presented. The reviewer has only some minor remarks for the text itself to be found below:

One major point, however, seems to be missing in the discussion: Does it really make sense to pyrolyse such highly valuable biological active and fertilizing materials like manure? It is well understood and shown that pyrolyzed manure has more nutrient related plant growth effects than pyrolyzed wood but what would be the result with composted manure? Or with liquid manure mixed to a wood based biochar? Although the authors did not compare non-pyrolysed manure in their trial, they should at least include this common practice and possibility into the discussion. The pyrolysis of manure volatilizes a major part of the organic nitrogen. Though even if it is emitted mostly as innocuous N_2, it is organic nitrogen that is lost from the nutrient cycle. The same counts though to a lesser extend for P, S and other minerals. It would be nice to include a nutrient mass balance or at least to mention it. Moreover, it is not clear if pyrolyzing manure is really advantageous from an economic point of view considering the high cost of pyrolysis devices and maintenance. The reviewer wants to suggest to include these reflections into the introduction, the discussion and to keep it in mind for conclusion.

Authors: We agree with the reviewer on the fact that we must mention these aspects and give briefly our opinion on this.

"We found results of some modeling exercises suggest that the moisture content of the feedstock plays a key role in determining the economic viability. In the case of swine manure the cost of solids separation (to 30% solids) followed by drying was found often excessive, whereas producing biochar from poultry litter (25% moisture) in an organic rankine cycle combined heat and power plant is a more potentially

interesting option. Some authors estimated that with a gate fee of €13/t the break-even selling point of the biochar would have been € 90/t (Huang et al., 2015, cited in the manuscript)."
However in several areas in Europe the interest in disposing these materials is so high that these solutions are still on the agenda. To progress we think that our experiment was anyhow necessary in order to verify the potential agronomic value of these materials and produce a clearer picture of advantages and disadvantages. Therefore if the question is in fact pyrolysis of manure could be a potential option in livestock manure processing, as a mean to stabilize organic matter in the feedstock and recover nutrients in the char. Now, we know that biochars mainly from poultry and to minor extent from swine manures, if pyrolysed at low temperature do have a significant fertilizing value.

As we didn't have a real data on how much nutrients got lost in the other byproducts (e.g. bio-oil and syngas) during pyrolysis. Instead, we have tried addressing this issue by mentioning the nutrient recovery from manure into the pyrolyzed manure based on previous studies. We cannot go into many details in this manuscript as was not the main hypothesis. This aspect would require a separate study that includes life cycle. However, in view of the comments made by the reviewer, we have thoroughly mentioned these aspects in our new Introduction (new pages 5-6), Discussion (new pages 17-18), and Conclusion (new pages 28-29).

Comment 2.2: Another import point is your consideration of biochar as "organic matter". It is clear that with the traditional combustion method for the analysis of SOC, there is no difference between SOC and biochar. But in biochar science, biochar is not considered as organic matter but as aromatic carbon, PyC or black carbon. If you add biochar to soil, it does not increase the humus content right away, this is only an analytical artefact.
Authors: We have carefully noted reviewer comment about misunderstanding between SOC and pyrogenic black C, and have corrected this throughout the text.

Specific Comments

Comment 2.3: Abstract: P2L26: if you mean the up-take by plants, please add this
P2L48: WC biochar could also work in combination with organic fertilizer not only NPK see e.g.
Authors: New page 2: Corrected the first part. We agree with the reviewer and have corrected this both in the abstract and the main text with inclusion of suggested references.

Comment 2.4: Introduction: P3L9: biochar is not considered as organic matter (see above)
P3L55 it is not proven yet what the crop growth promoting factors really are. Please write rather: …amended soils is thought to be enhanced…
Authors: New page 3: We agree with the reviewer as the biochar C is recalcitrant. We have replaced the organic matter with "pyrogenic material". We have corrected for the both comments.

Comment 2.5: P4L19: There is plenty of literature about pyrolyzed manure. Please check literature.
Authors: New page 4: We have restructured following the suggestion from the reviewer (see page 4, 1st paragraph).

Comment 2.6: P4L24: What is wet pyrolysis? How should that work?
Authors: New page 4: Actually wet pyrolysis (in presence of water/steam) and hydrothermal carbonization (HTC) are the same. We have corrected this in our text.

Comment 2.7:P4L36: delete (aromatic) or include it into the sentence
P5L21: often been less, but sometimes more? ;-) Please delete "often"
Authors: New page 4: Corrected as suggested. We have included this word 'aromatic' in the sentence.

Comment 2.8: P5L34: see above, evaporation of manure water (and N) is no major cost benefit…
Authors: New pages 5-6: We agree with the reviewer and have considered this issue in our new text (see page 6: 2nd paragraph: last sentence).

Comment 2.9: P6L21: The reviewer is not happy with how the analytical methods in table 1 and table 2 are described. I would suggest a more detailed description but leave it to the editor if he accepts it like this.
Authors: New pages 7-8: We agree with the reviewer and this comment is also raised by the reviewer #1. We have now expanded these sections (2.1 and 2.2-second paragraph) elaborating the details of each analysis by providing the references.

Comments 2.10:P7L4: please write kiwi tree not kiwi fruit
P13L33: Please see above, don't confound SOC and BC
Authors: New pages 8 & 14: Both corrected as suggested.

Comment 2.11: P16L43: Bargmann et al published on HTC chars and Deenik on sewage sludge chars which are very different materials than certified biochars.
Authors: New page 18: We agree with the reviewer therefore our new sentence is expanded and we give details on the type of biochars included in these studies (page 18, 3rd paragraph, last sentence).

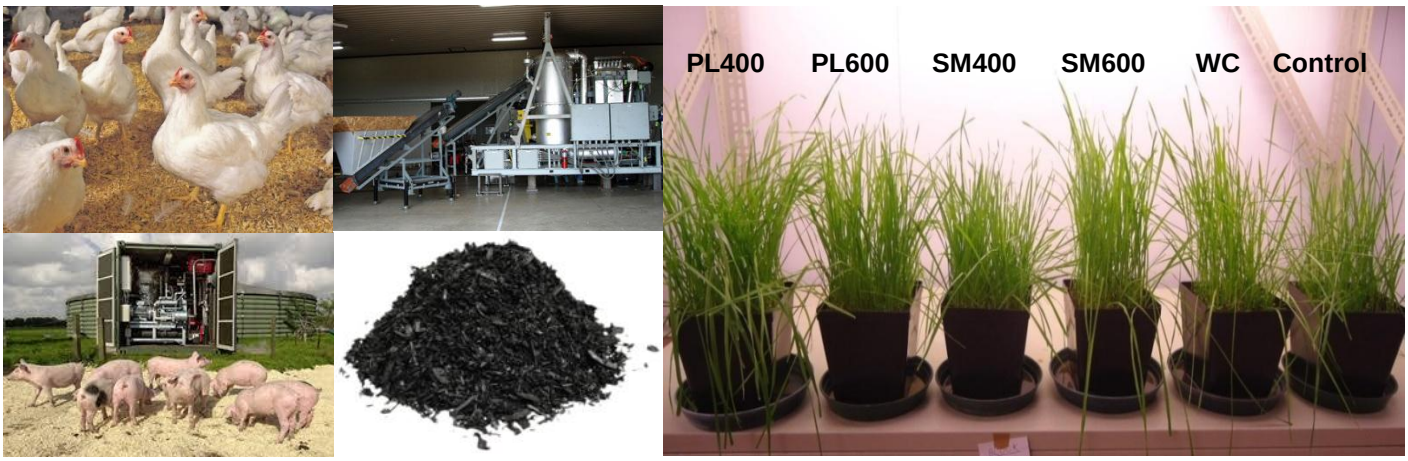
Comments 2.12:P17L9: write: may be due instead of "is due"
P19L31: we can only exclude major negative effects of heavy metals. You cannot exclude any effect…
P19L46: see above, don't confound SOC and BC
Authors: New pages 19 & 21: All corrected as suggested. Following the suggestions of reviewer #1 on heavy metal content of biochar, we now have compared our results with the official threshold values set by the European Biochar Certificate & International Biochar Initiative. Our values are under such thresholds. We deleted all other comments.

Comment 2.13: P25: The conclusion is rather long and repetitive.
Authors: New pages 28-29: We have now refined and shortened conclusion within 265 words by highlighting the main outputs and avoiding repetition.

Comment 2.14: P25L34: Please consider here the above suggested addition in the discussion of nutrient balances between manure and pyrolysed manure.
Authors: New pages 28-29: We now have refined our conclusion mentioning the nutrient recovery from manure into the pyrolyzed manure (char) and importance of economic assessment of manure pyrolysis too. We couldn't consider a real/actual mas balance in our study as we didn't have a real data on how much nutrients got lost in the other byproducts (e.g. bio-oil and syngas) during pyrolysis (this would be a separate study).

Comment 2.15: P26L21: Please consider to add "potential" before "excessive nutrient load". You could adapt the application amounts and you did not investigate the leaching…
Authors: New page 29: Corrected.

***Graphical Abstract**



Picture 1. Graphical abstract highlighting "Chemically and biologically-mediated fertilizing value of manure-derived biochars".

HIGHLIGHTS

- Low temperature manure-derived biochars enhanced both crop yield and NPK uptake, and improved soil properties.
- Manure biochars showed more positive effects on acidic silt-loam soil than on alkaline sandy soil.
- Wood chip biochar had no effect on crop yield, but showed a good C sequestration potential.
- All biochars shifted bacterial community structure and modified enzyme activities.

Chemically and biologically-mediated fertilizing value of manure-derived biochar

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HIGHLIGHTS

- Low temperature manure-derived biochars enhanced both crop yield and NPK uptake, and improved soil properties.
- Manure biochars showed more positive effects on acidic silt-loam soil than on alkaline sandy soil.
- Wood chip biochar had no effect on crop yield, but showed a good C sequestration potential.
- All biochars shifted bacterial community structure and modified enzyme activities.

Key words: Pyrolysis temperature, amendment, fertilizer, crop growth, carbon sequestration, soil enzymes activity

ABSTRACT

This study evaluates the potential of manure-derived biochars in promoting plant growth and enhancing soil chemical and biological properties during a 150 day pot experiment. Biochars from pyrolysis of poultry litter (PL) and swine manure (SM) at 400 and 600°C, and a commonly available wood chip (WC) biochar produced at high temperature (1000 °C) were incorporated to silt-loam (SL) and sandy (SY) soils on a 2% dry soil weight basis. Ryegrass was sown and moisture was adjusted to 60% water filled pore space (WFPS). The PL400 and SM400 biochars significantly increased (p<0.05) shoot dry matter (DM) yields (SL soil) and enhanced nitrogen (N), phosphorus (P) and potassium (K) uptake by the plants in both soils, compared to the Control. All biochars significantly increased the soil carbon (C) contents compared to the Control. Total N contents were significantly greater for PL400 and PL600 treatments in both soils. The dehydrogenase activity (DA) significantly increased for PL400 and SM400 treatments and was positively correlated with the volatile matter (VM) contents of the biochars, while β-glucosidase activity (GA) decreased for the same treatments in both soils. All biochars significantly shifted (p≤0.05) the bacterial community structure compared to the Control. This study suggests that pyrolysis of animal manures can produce a biochar that acts as both soil amendment and an organic fertilizer as proven by increased NPK uptake, positive liming effect and high soil nutrient availability, while WC biochar could work only in combination with fertilizers (organic as well as mineral).

Introduction

Biochar, a carbonaceous solid recalcitrant pyrogenic material, has drawn considerable scientific attention due to its potential in climate change mitigation, waste management, soil fertility enhancement and crop growth promotion (Chan et al., 2007; Hossain et al., 2011; Ameloot et al., 2014; De La Rosa et al., 2014; Jeffery et al., 2015). The environmental benefits of biochar application to soils are associated with increased carbon (C) sequestration (Lin et al., 2015) and suppression of greenhouse gas (GHG) emissions (Kammann et al., 2012), while agronomic benefits include improved soil structure and porosity, increased surface areas, water holding and cation exchange capacities (CEC) of biochar amended soils (Lehmann et al., 2003; DeLuca et al., 2009; Cornelissen et al., 2013). However, there still remains some dispute regarding biochar use in soils as few reports comment on their negative effects on to crop growth (Deenik et al., 2010; Wisnubroto et al., 2011), due to toxic volatile compounds that are potentially formed during pyrolysis (Spokas et al., 2011), and these could also eventually affect the soil microbiota (Gul et al., 2015).

The biochar-amended soil nutrients (both micro and macro) availability depends on many physico-chemical char properties, such as pH, surface area (SA), porosity, CEC and the transfer of nutrients into the amended soil (DeLuca et al., 2009; Clough et al., 2013). In addition, manure biochars also have potential as liming agent for acid soils because of their high ash contents (Srinivasan et al., 2015; Subedi et al., 2016). The crop growth promotion in biochar amended soils is thought to be enhanced nutrient use efficiency in addition to reduced leaching associated with positive enhancement of soil chemical (Lehmann et al., 2003; Knowles et al., 2011; De La

Rosa et al., 2014), and microbial properties (Nielsen et al., 2014, Gul et al., 2015). Low nutrient content biochars produced mainly from wood biomasses, also known as charcoal, show positive effects on crop growth only when applied in combination with fertilizers (Steiner et al., 2007, Deenik et al., 2010; Subedi et al., 2015; Schmidt et al., 2015). In contrast, manure biochars may also act as biofertilizers as their original feedstocks are rich in nutrients (Singh et al., 2010; Hossain et al., 2011; Cantrell et al., 2012), with limited information on their agronomic value and thus seeking for further experimental evidence.

The stability of biochar in soils depends on several concurrent factors such as feedstock quality, type of thermal process (low/high temperature pyrolysis, slow/fast pyrolysis, gasification, hydrothermal carbonization), the processing conditions (temperature and residence time), the resulting biochar properties as well as the environmental factors (Zimmermann et al., 2011; Kammann et al., 2012; Ameloot et al., 2014; Gul et al., 2015; Subedi et al., 2016). The recalcitrant biochar carbon and its aromatic structure might interfere with the natural environment of soil organic matter and affect microbial diversity, abundance and community composition (Lehmann et al., 2011). The highly porous structure of biochar can provide protection and an aerated habitat for the mycorrhizal fungi and bacteria, potentially changing the bacterial biogeochemical nitrogen (N), phosphorus (P) and sulphur (S) cycles, and their community composition (O'Neill et al., 2009; Fox et al., 2014; Schmalenberger and Noll et al., 2014). These changes are among the drivers for plant growth promotion based on evidences of nitrification, N-fixation, P- and S- mobilization by the respective functional bacteria (Lehmann et al., 2011; Fox et al., 2014).

Biochar's effect on soil microbial biomass has been widely reported (Lehmann et al., 2011; Gul et al., 2015). As biochar can modify soil properties such as soil structure, pH, CEC and the availability of organic C, it will influence the size of the microbial biomass (Cheng et al., 2008; Smith et al., 2010; Lehmann et al., 2011; Bruun et al., 2011). The amount of labile C from the biochar is of particular importance as this provides a readily available carbon energy source that increases microbial decomposition and finally microbial biomass (Gonzalez-Quiñones et al., 2011). Biochar can also have a major effect on soil microbial enzymatic activity (Ameloot et al., 2014, 2015). Enzymes such as dehydrogenase (intracellular) and β-glucosidase (extracellular) represent the indicators of soil microbial activity in assessing the degree of resistivity of organic matter (OM) in biochar-amended soils against microbial degradation. The activities of these enzymes are thus influenced by the nature of C compounds (labile and/or aromatic) present in the biochar (Camina et al., 1997), the pyrolysis temperature, the nature of the enzyme itself, and the incubation time (Wang et al., 2015b).

Manure may not be an ideal feedstock for thermal treatment, such as combustion and gasification at high temperature, due to its high moisture and alkali metal content, causing ash agglomeration (Di Gregorio et al., 2014; Lynch et al., 2013). Pyrolysis operates at lower temperatures (300–600 °C) compared to combustion and gasification, reducing the risk of ash agglomeration and harmful emissions during combustion (NO_x, SO_2, particulates) (Basu, 2010). Nevertheless, from a waste management perspective there are good reasons why manure should be addressed to thermal treatment, particularly as recent EU legislations are trying to enforce a more sustainable agricultural system. The main advantages are a reduction of waste in volume

and mass, reduction of pathogens and odour compounds, reduced nutrient runoff and added income from selling ash, char and gas (Arena, 2012).

The potential agronomic and environmental benefits of producing biochar from manure, and the possibility of using it as a soil amendment has been less explored compared with the commonly available biochars produced from wood biomasses, and is of scientific interest in the manure management chain (Sing et al., 2010; Cantrell et al., 2012; Jensen, 2013b). Moreover pyrolysis could be an option to stabilize OM in the manure feedstock and recover most of the nutrients in the mineral ash fraction, despite significant cost of this process and the N and S losses are unavoidable (Cantrell et al., 2012; Jensen, 2013a; Huang et al., 2015).

A previous experiment demonstrated high microbial respiration in soil, high N mineralization potential and high availability of N, P, K and other cations as well as the liming potential of manure-derived biochars (Subedi et al., 2016). We opted to further explore these findings by showing that manure-derived biochars have the potential to sustain crop growth due to induced physical, chemical and biological changes in soil. We therefore continued the previous experiment using the same experimental substrates under controlled environmental conditions, with the following hypotheses: (i) low temperature pyrolysis of manure feedstock produces biochar with a high fertilizing value and low toxicity, (ii) high temperature pyrolysis is effective in producing a recalcitrant amendment, and (iii) pyrolysis conditions (temperature and feedstock) modulate the effects on residual soil chemical and microbial properties.

2. Materials and methods

2.1. Soil collection and characterization

The two soils used in this experiment had contrasting characteristics (Table 1): i) a silt-loam soil, referred as "SL", high in OM with slightly-acidic pH, and ii) a sandy soil, designated as "SY", low in OM with moderately alkaline pH (Subedi et al., 2016). The two soils were taxonomically classified as Alfisol (SL soil) and Entisol (SY soil) according to the USDA soil classification system (USDA, 1999). Both soils were low in N, P and K contents, but rich in Ca contents. They were both collected from the top 20 cm plough layer of arable fields (NW Italy). The soils were then air-dried and mechanically sieved (2 mm particle size), using an electric auto-rotating sieving device (Neotron s.r.l., Autopack, Modena, Italy). Soil texture was determined by the pipette method (Gee and Bauder, 1994), soil bulk density by the core method and particle density by the pycnometer method (Blake and Hartge, 1994). Total pore volume was calculated based on the bulk density and particle density values obtained. The soils were analysed for total C and N contents using a total elemental analyser (Vario El Cube, Elementar, Hanau, Germany). The chemical analysis (pH, Olsen P, K, Ca, Mg and CEC) was carried out following routine analytical procedures (Sparks, 1996).

2.2. Biochar production and characterization

The biochars used for this experimental study were produced from two different manure feedstocks (poultry litter (PL) and swine manure (SM)) at two different pyrolysis temperatures (400 and 600 °C) (Table 2). The poultry litter biochars ("PL400" and "PL600") were produced at

the University of Limerick (Ireland), using a laboratory pyrolysis plant, while the swine manure biochars ("SM400" and "SM600") were supplied by ECN in the Netherlands (www.ecn.nl). Another widely available commercial biochar from wood chip (WC) was included as a reference material. This was produced from kiwi tree pruning residue via industrial gasification (1000 °C) at Agrindustria, Italy (www.agrind.it). A biochar-free control completed the experiment.

Biochars were physically characterized for surface area (SA) and pore volume (PV) according to the Brunauer, Emmet and Teller (BET) method, via the measurements obtained by N_2 adsorption at 77K using an ASAP-2400 Micrometrics apparatus (Table 2). They were chemically characterized for total C, N, available P (2% formic acid extractable), cations (K, Ca and Mg), pH and CEC as mentioned in Subedi et al. (2016). The total P and heavy metal contents were analysed by Atomic Absorption Spectroscopy (Varian Techtron AA6, Melbourne, Australia), following acid digestion of biochar samples (Cantrell et al., 2012). Proximate analysis was carried out for the determination of the ash and volatile matter (VM) contents according to the NSAI standard testing method (NSAI, 2009). Each measurement was carried out in triplicate. As reported by Subedi et al. (2016), with respect to WC biochar, all manure biochars were higher in nutrient contents, VM and CEC, lower in C contents, surface areas and pore volumes (Table 2). Except for Al and Pb, the heavy metal concentrations for the manure biochars were also higher compared to the WC biochar.

2.3. Plant growth experiment setup

The growth experiment was organized in a completely randomized design with three replicates in a controlled climatic chamber (20 ºC, 65% relative humidity and photon flux of 260 µmol m^{-2} s^{-1}). The soil used in this experiment derived from the previous one started 9 months before, when the two soils were air dried, sieved (2 mm), amended with biochar (2% w/w), rewetted and fertilized with NH_4NO_3 (>99% purity, Merck KGaA, Darmstadt, Germany) at 170 kg N ha^{-1}, as fully described in Subedi et al. (2016).

After pooling the replicates from that experiment, amended soils were repacked into plastic pots (1.5 kg for each replicate, d=13.5, h=13.5 cm), and moistened to 60% water filled pore space (WFPS) using normal irrigation water (<6 mg l^{-1} NO_3^--N, negligible PO_4^{3-}). Soils were allowed to stabilize for a week then, seeds of Italian ryegrass (*Lolium multiflorum L.*) were sown (0.2 g pot^{-1} given the seed rate of 10 g m^{-2}) on the soil surface (0.0182 m^2) and gently pressed with finger to ensure maximum soil-seed contact. The soil water content was adjusted every three days by weighing the pots and adding water to achieve the original moisture content. No extra fertilization was undertaken after sowing. A total of five harvests were completed during the entire growth period of 150 days and dry matter (DM) yield was recorded.

2.4. Plant and soil analysis

Each of the five harvests was performed by cutting the above ground biomass approximately 2 cm above the soil surface, dried at 40 °C for 72 h and dry weights were recorded. After completion of the growth experiment, roots were carefully hand separated from the soils, thoroughly washed with water and dried using the same procedure as for the shoots. Total shoot

N content was determined by a total elemental analyser (Vario El Cube, Elementar, Hanau, Germany). Total shoot P and K contents were analysed by calcination followed by acid recovery using inductive coupled plasma mass spectrometry (iCAP^{TM} Q ICP-MS, Thermo Scientific^{TM}, Pittsburgh, USA). The NPK uptakes were calculated as a product of tissue NPK concentration and biomass yield. The biochar NPK uptake efficiencies (UEs) were also calculated from the increase in uptake between the Control and biochar treatments (Equation 1), and expressed as percentage of NPK supplied from the biochars as described by Jensen (2013b).

$$UE = \frac{Uptake\ (treat.) - Uptake\ (ctrl.)}{NPK\ supplied\ via\ biochar} \times 100\% \quad (1)$$

Where,

UE = 'N' or 'P' or 'K' uptake efficiency (%),

Uptake (treat.) = NPK uptake (mg pot^{-1}) with biochar treatments,

Uptake (ctrl.) = NPK uptake (mg pot^{-1}) with the Control treatment, and

NPK supplied = Total N or P or K (mg pot^{-1}) supplied to the soil from the respective biochars.

At the end of the growth experiment, the biochar-amended soils were characterized both chemically and biologically. The soils were analysed for total C and N contents using a total elemental analyser (Vario El Cube, Elementar, Hanau, Germany), while pH, available P (Olsen) and CEC following routine analytical procedures (Sparks, 1996). The soil mineral N was analysed colorimetrically following the procedure in Subedi et al. (2016). Soil microbial biomass carbon (MBC) was determined by the fumigation-extraction method (Beck et al., 1997). The dehydrogenase activity (DA) was determined following a procedure described in Camina et al.

(1997). The protocol for β-glucosidase activity (GA) was adapted from Eivasi and Tabatabai (1998). Soils were stored at 4 ºC for MBC and enzyme determination and at -25 ºC for DNA extraction until analysis.

2.5. Soil DNA extraction and measurement, and bacterial community structure analysis

Soil DNA was extracted from the bulk soil following a protocol of the POWER SOIL® DNA isolation kit (MO BIO Laboratories, Cupertino, CA, USA) as per guidelines from the manufacturer. The extracted DNA was quantified spectrophotometrically (NanoDrop ND 1000, Thermo Scientific, Pittsburgh, USA). The 16S rRNA gene from 5-10 ng template DNA was subsequently amplified using the universal primers 348GC and 518R (Muyzer, 1993) with a touch-down polymerase chain reaction (PCR) protocol as outlined previously (Fox et al., 2014). Subsequent soil bacterial community structure analysis was carried out via denaturing gradient gel electrophoresis (DGGE) at a gel strength of 10 % with a 35 to 65 % denaturing gradient and electrophoresis was conducted for 1040 Vh at 60°C in TAE buffer (Fox et al., 2014). Gels were stained after electrophoresis with SybrGold (Invitrogen, Carlsbad, CA) and DNA bands were visualized in a UV transilluminator (G:Box, Syngene, Cambridge, UK). Phoretix 1D was used to analyse the DGGE profiles and to create a binary matrix (Totallab, Newcastle upon Tyne, UK).

2.6. Data processing and analysis

All data concerning ryegrass yield, nutrient uptake and soil properties were analysed using a one-way ANOVA (IBM SPSS statistics 22) separately for each soil type. Statistical significance

was tested at $p<0.05$. The validity of model assumption for each variable was verified by examining the residuals for normal distribution (Shapiro-Wilk test) and homoscedasticity (Levene's test). Data violating the model assumptions were logarithmically transformed, analysed by ANOVA and the back transformed values to the original scale were reported. A common standard error (SE) of the mean from the pooled variance, for each measured variable, was reported for all the treatments. Tukey's HSD post-hoc test was applied for pairwise comparison to assess any significant differences ($p<0.05$) between treatment means. Additionally, a Pearson bivariate correlation analysis was performed for the different measured variables to assess any correlation of the biochar properties with ryegrass yield, NPK uptake, and soil microbial properties.

Binary matrices from 16S rRNA gene fragment based DGGE were analysed via canonical corresponding analysis (CCA) alongside environmental factors (e.g. soil pH, SOC, TN, C/N ratio and ryegrass yield) using CANOCO (Microcomputer Power Inc., Ithaca, NY). Permutation tests were conducted (9,999 repeats) to identify differences of DGGE profiles at significance level ($p\leq0.05$) as described by Noll (2008).

3. Results

3.1. Ryegrass yield, nutrient uptake and efficiency

Results from ryegrass growth demonstrated that the low temperature manure-derived biochars (both PL400 and SM400) significantly increased both shoot and root DM yield compared to the

Control in the SL soil, while PL600, SM600 and WC biochars had no effect on either shoot or root DM production (Fig. 1). Only the PL400 treatment significantly increased shoot and root DM in SY soil. The highest shoot DM yield increase of 50.1% compared to the Control was recorded in PL400 treated SL soil, followed by 44.0% with PL400 treated SY soil. Similarly, up to 127.2% increase of root biomass (DM) compared to the Control was observed for PL400 treated SL soil followed by 93.8% increase with SM400 treatment. The biochar N content was significantly positively correlated with both the shoot DM yield (n=15, $p<0.01$, r=0.78 for SL and $p<0.05$, r=0.61 for SY) and the root DM yield (n=15, $p<0.01$, r=0.82 for SL and $p<0.05$, r=0.59 for SY).

The N uptake significantly increased compared to the Control and WC for the PL400 and SM400 treatments in the SL soil, while this was significantly increased for all manure biochar treatments in the SY soil (Table 3). The highest increase in N uptake by 64.4% compared with the Control was recorded in PL400 treated SL soil followed by 40.4% increase in SM400 treated in the same soil. The P uptake was significantly enhanced for all manure biochar treatments compared with the Control and WC biochar in SL, while only PL400 and SM400 treatments increased P uptake in the SY soil. The increase in P uptake up to 161% compared to the Control was recorded in PL400 treated SL followed by 119% with the SM400 in the same soil. Similarly, K uptake was significantly enhanced for all manure biochar treatments compared to the Control in the SL soil, while only PL400, PL600 and SM400 treatments increased K uptake in the SY soil. Up to 210% increase in K uptake compared with the Control was observed in PL400 treated SL soil followed by 142% increase in PL400 treated SY soil. The WC biochar had no effect on NPK uptake compared to the Control.

The N UEs, with respect to the total N supplied via biochar, for PL400 and SM400 treatments were greater compared with the other biochar treatments in the SL soil, while higher efficiency was recorded for only SM400 treatment in the SY soil (Table 3). The P UEs for PL400 and SM400 treatments were greater compared with the other treatments in both soils, with the highest value for PL400 treated SL soil followed by SM400 treatment in the same soil. In contrast to these, the highest K UE was recorded in the WC treated SL soil followed by SM400 treatment in the same soil, and the lowest K UE for the SM600 treated SY soil. The NPK UEs, over all treatments, in the SL soil were two times greater than in the SY soil. Significant positive correlations existed between ryegrass N uptake and biochar N contents (n=15, $p<0.01$, r=0.65 for SL and r=0.71 for SY), P uptake and biochar P contents (n=15, $p<0.01$, r=0.68 for SL and $p<0.05$, r=0.50 for SY), as well as K uptake and biochar K contents (n=15, $p<0.01$, r=0.66 for SL and r=0.70 for SY).

3.2. Soil chemical properties

After the last ryegrass harvest, significant effects on all soil properties as a result of biochar additions to both soils were observed apart from soil CEC. The increase of soil C content from a mean of 1.29% in the Control to 2.74 % in the WC treatment was recorded in the SL soil, while the increase remained between 0.91 (Control) and 2.72% (WC) in the SY soil (Table 4). The highest increase of soil C content by 198% compared to the Control was calculated for the WC treated SY soil followed by a 116% increase for the SM400 treated same soil. The mineral N concentrations in both soils after ryegrass harvest were very low (3 mg N kg^{-1}, on average), without differences among treatments. Similarly, a significant increase in the total soil N content

from a mean of 0.13% in the Control to 0.23% in the PL400 treatment was noticed in the SL soil, while such an increase was observed between 0.06% (Control) and 0.15% (PL400) in the SY soil. The WC biochar had no effect on the total N content compared to the Control in either soils. All manure biochars significantly increased the soil pH compared with the Control, while WC had no effect on pH in both soils. An increase in pH from 6.5 pH unit in the Control to 7.88 pH unit in PL600 and SM400 was observed in the SL soil, while pH varied between 8.59 and 9.21 in the SY soil.

Manure biochars caused a significant increase in available P, from a mean value of 20.8 mg kg^{-1} for the Control to a mean of 106.4 mg kg^{-1} for the SM600 treatment in the SL soil (Table 4). The P content in the SY soil was also significantly higher for the manure biochars compared with the Control and WC treatments. Exchangeable K increased significantly only for the PL600 and SM400 treatments in the SL soil, however, while all manure biochars increased exchangeable K in the SY soil. Both exchangeable Ca and Mg significantly increased for all manure biochar treatments in the SL soil. In the SY soil, none of the biochars had an effect on exchangeable Ca, instead a slow steady decline in Ca content was observed relative to the Control, however exchangeable Mg increased significantly with the addition of manure biochars. The CEC failed to vary significantly among the biochar treatments in either soils (Table 4).

3.3. Soil microbial properties and community structure

In the SL soil, the MBC values were significantly lower (p<0.05) compared with the Control for the PL600, SM400 and SM600 treatments, while they were significantly higher for all

biochar treatments including WC in the SY soil (Table 4). The MBC values, on average over all treatments, did not differ between two soil types. The DA significantly increased compared with the Control for the PL400, SM400, SM600 treatments in the SL soil, while this was true only for the PL400 treatment in the SY soil. On the other hand, significantly lower DA for WC treatment compared to the Control and manure biochar treatments was observed in the SL soil, however in the SY soil, this was true for the WC, PL600 and SM600 treatments. The average DA value, over all treatments, was 1.8 times higher in the SL soil than in the SY soil. The DA, in both soils, was significantly positively correlated with the VM contents of the biochars (n=15, $p<0.05$, r=0.61 for SL and $p<0.01$, r=0.91 for SY), but negatively correlated with the biochar C:N ratios (n=15, $p<0.01$, r= -0.82 for SL and r= -0.80 for SY).

The GA was found to be significantly lower for the PL400 and SM400 treatments compared with the Control and WC treatments in the SL soil, and this was also true for the PL400, PL600 and SM400 treatments in the SY soil. Significant negative correlation was observed between GA and the VM contents of the biochar (n=15, $p<0.05$, r= -0.61 for SL and $p<0.01$, r= -0.68 for SY), but a positive correlation between GA and the biochar C:N ratios (n=15, $p<0.01$, r=0.81 for SL and r=0.67 for SY). Over all treatments, GA was on average 1.6 times lower in the SL soil than in the SY soil. The correlation between DA and GA was significantly negative (n=18, $p<0.01$, r= -0.84 for SL and $p<0.05$, r= -0.53 for SY). Moreover, soil pH was positively correlated with DA but only in the SL soil (n=18, $p<0.05$, r=0.57), but was negatively correlated with GA in both soils (n=18, $p<0.05$, r= -0.55 for SL, $p<0.01$, r= -0.65 for SY).

Permutation testing of the bacterial DGGE community profiles revealed a significant separation between the Controls and all biochar treatments in both soils ($p \leq 0.05$). Furthermore, significant differences were found in SL between treatments WC and SM, WC and PL, as well as PL and SM. However, significance was not reached between the two different manure pyrolysis temperatures 400 and 600°C. (Fig. 2a and 2b). In SY, significant differences between the two PL and between PL and WC were found, SM was not significantly different from WC and no significant difference was found between the two SM treatments. Canonical correspondence and permutation analysis of SL identified a significant influence of the soil pH, SOC, TN, C/N ratio and shoot DM yield on the bacterial community structure. Likewise, the same significant influences were identified in SY, with the exception that variations in SOC wasn't significantly influencing the bacterial community structure (Fig. 2a and 2b, arrows indicate significant environmental factors).

4. Discussion

The production of biochars from the manure is of scientific interest as a mean to stabilize the OM in the feedstock, especially where there is an over application of manure and digestates; land application is restricted due to excessive amount of nutrients already present in the soil and their export is limited due to the national legislation. The technical and economic feasibility of producing biochar from pre-treated swine manure and poultry litter has been modelled and simulated based on experimental data (Wnetrzak et al., 2013; Huang et al., 2015). The results of these modelling exercises suggest that the moisture content of the feedstock plays a key role in determining the economic viability. Poultry litter can be supplied to the reactor directly without

any pre-treatment because the relatively low moisture content of such feedstock makes it suitable for pyrolysis (Lynch et al., 2013; Huang et al., 2015). Pig slurry, on the other hand, has to be separated into solid and liquid fractions, as it has a very low dry matter content (Wnetrzak et al., 2013).

At operating temperatures between 300°C and 700°C, most inorganic compounds including essential plant nutrients such as P, K, Ca, Mg etc. can be recovered (100–80%) in the char, even though the loss of some N and S is unavoidable (Cantrell et al., 2012; Van Zwieten et al., 2013). Previous studies reported that the recoveries for C (70–30%), N (75–20%) and S (65–20%) vary within the pyrolysis temperatures (300–700 °C) and among the type of manure feedstocks (e.g. poultry/turkey litter, swine/dairy/horse manure etc.) (Cantrell et al., 2012).

This study showed a remarkable and positive effect of low temperature poultry biochar, both on crop growth and soil chemical properties and subsequent alterations in soil microbial properties. To a minor extent, this was also true for the SM400 but only in the finer textured SL soil. The results of biochar response to crop growth from previous studies are quite variable but a majority of them reported positive yield enhancement (Chan et al., 2007, Van Zwieten et al., 2010; Uzoma et al., 11; Wang et al., 2012b; Zhang et al., 2012; Cornelissen et al., 2013, De La Rosa et al., 2014, Fox et al., 2014, Lin et al., 2015), some studies with no effect at all on yield (Bargmann et al., 2013, Cornelissen et al., 2013, Lin et al., 2015), and a few studies showed negative effects on plant growth, such as Deenik et al., 2010 on macademia nut-shell charcoal, Wisnubroto et al., 2011 on sewage-sludge biochars and Bargmann et al., 2013 on hydrochar. The same biochar used in the same soil in an experiment for two different crops can achieve different

results on crop growth performance as Lin et al. (2015) reported increased wheat yield on maize stalk biochar amended loamy soil (16 Mg ha^{-1}), but found no effect on soybean growth for the identical treatment. This also suggests that crop response to biochar varies with crop types, biochar application rates and properties, growing conditions and edaphic factors (Jeffery et al., 2011).

The higher DM (both shoot and root) yield of ryegrass, in both soils, particularly from low temperature manure biochar treatments may be due to: (i) direct nutrient additions from these chars into the soil-biochar matrix (Lehmann et al., 2003), (ii) higher N mineralization from these biochar amended soils (Ameloot et al., 2015), (iii) improved bioavailability of soil-biochar-P associated with increase in soil pH (in the SL soil) followed by enhanced P uptake plus efficiency (Wang et al., 2012b; Fox et al., 2014), and (iv) improved soil biogeochemical S cycling attributed to the activity of soil biota (Lehmann et al., 2011, Fox et al., 2014; Subedi et al., 2016).

We observed increased N uptake followed by an enhanced N uptake efficiency in the ryegrass plants for the low temperature manure biochar treatments in the SL soil and with all manure biochar treatments (except SM600) in the SY soil. Clough et al. (2013) reported that the low temperature manure- and biosolids-derived biochars contain substantial amount of hydrolysable organic N such as amino acids that could be mineralized microbially, thus would become plant available indirectly or could be even taken up by plant roots directly. This hydrolysable N decreases with increasing pyrolysis temperature (Hossain et al., 2011; Wang et al., 2012a). The positive correlation between N uptake and biochar N content suggested that a substantial

proportion of N from these chars was indeed ultimately plant available (De la Rosa and Knicker, 2011). Additionally, increase in shoot and root DM yield for PL400 and SM400 treatments compared with the Control was accompanied by the enhanced N uptake in the respective treatments in the SL soil. On the other hand, a significant increase in N uptake for PL600 and SM400 treatments, in the SY soil did not necessarily increase shoot and root DM yield compared with the Control. This indicates that soil characteristics also play a role and biochar is more effective in an acidic soil because of its neutralization effect (Deenik et al., 2010; Fox et al., 2014; Lin et al., 2015). Furthermore, the low CEC and OM as well as strong soil alkalinity could be the additional causes for the lack of DM response to the SY soil, leading to restricted plant growth.

We further noted that the causality of biochar derived plant growth promotion was not solely determined by the N uptake but also included enhanced P and K uptake (Van Zwieten et al., 2013), as well as other beneficial cations (Ca, Mg etc.) as significant positive correlations also existed between P, K uptake and biochar P, K contents. The significant increase in P uptake along with the positive correlation corroborates that biochar P is directly or indirectly available to the crops (Wang et al., 2012b; Van Zwieten et al., 2013). Like for N, the higher P UE with PL400 and SM400 treatments in the SL soil also indicates that available fraction of P, associated with enhanced mineralization/mobilization of organic P, is greater in low temperature manure biochars than in the high temperature biochars (Van Zwieten et al., 2013; Jensen, 2013a, 2013b).

The WC biochar in this study did not show an improvement in plant growth, but on the other hand did not reduce the growth with respect to the Control. This indicates that this commercial

biochar could show a positive effect on C sequestration but it needs NPK fertilization when applied to crop (Steiner et al., 2007; Deenik et al., 2010; Van Zwieten et al., 2010; Subedi et al., 2016). Nutrient poor biochars may still act as soil conditioners, altering soil chemical and soil microbial properties resulting in increased soil nutrient uptake that were not available in otherwise acidic soils (Van Zwieten et al., 2010; Lehman et al., 2011; Fox et al., 2014; Gul et al., 2015). On the other hand, the PL400 biochar used in this study had the highest VM and N contents, and the lowest C:N ratio of all biochars. Thus, the N mineralization on such biochar-amended soil is also expected to be higher due to the low C/N ratio and this was fully evidenced by the higher N uptake. The harmful effect of manure biochars due to their high VM contents on ryegrass growth was not visible in our study, in contrast with previous studies which found reduced lettuce and maize growth on macadamia nut shell charcoal-amended soil (Deenik et al., 2010), reduced ryegrass growth on biosolid biochar amendments (Wisnubroto et al., 2011) and inhibited wheat seed germination on hydrochar amended soils (Bargmann et al., 2013). This suggests that not all biochars are harmful to the crops, toxicity being determined only by specific combination of feedstock and processing conditions (Spokas et al., 2011). We also noticed higher concentrations of heavy metals (Zn, Cu, Mn and Ni) in the PL biochars compared with other biochars (Table 2). Nevertheless, the heavy metal concentrations for all biochars used in our study are below the range of maximum threshold values set by different biochar certification schemes (EBC, 2012; IBI, 2014; Domene et al., 2015).

The increase in nutrient (both macro and micro) contents of biochar-amended soils is mainly associated with the direct addition of these elements (DeLuca et al., 2006; Subedi et al., 2016). An expected significant increase in soil C contents with biochar additions in both soils showed

the potential of such biochars in sequestrating C in the long run. Such a large increase in soil C contents can also be explained due to the higher root biomass turnover associated with increased biomass production as result of soil biochar addition (Kammann et al., 2012). A significant increase in the total N contents with the PL400 and PL600 treatments showed that this N could successively be available, via microbial mineralization, for the subsequent crops (Clough et al., 2013). However, the mineral N fraction of all treatments were too low at the end of the ryegrass harvest, indicating that the plants were able to up take almost all of the available N forms.

All the biochars significantly increased the soil pH compared with the Control in both soils. The increase in pH of the slightly acidic SL soil is a positive liming effect towards soil neutralization (Fox et al., 2014); however this pH increase in the already alkaline SY did not play any negative role in terms of ryegrass DM yield. None of the biochars in this study improved soil CEC. Even though the contact between soil and biochars was at least 1 year (Subedi et al., 2016), they were not successful in enhancing the CEC. Organic matter in biochar feedstock often loses its functional groups during pyrolysis and they can rebuild again over time as result of biochar oxidation into soils, but this may take several years to see the actual effect on soil CEC (DeLuca et al., 2006, Singh et al., 2010).

The available P contents in soil-manure biochar matrix in both soils, after ryegrass harvest, were significantly higher compared with the Control providing further evidence that a large fraction of biochar P was available to the crop and these materials can also act as a P fertilizer (Wang et al., 2015a). We can also notice that the significant amount of residual P after ryegrass harvest may also lead to P overload in the soil eventually contributing to environmental problems

(e.g. eutrophication, leaching). The exchangeable K contents was significantly higher than the Control only for the PL600 and SM400 treatments in the SL soil, while it was higher for all manure biochar treatments in the SY soil. The average K content, over all treatments, in SL soil was 1.6 times lower than in the SY soil and was linked to the high K uptake in the SL soil. We noticed that the plant was able to deplete more K from the SL soil than from the SY soil as SL soil had high DM yield. Furthermore, Ca may have competed more with K for the exchange sites in the SL soil (except PL600 treatment) due its higher affinity as the Ca availability is relatively higher in SL soil than in SY soil, while opposite can be true for SY soil (Sachs, 2004). It is also true that a near neutral pH normally reduces the amount of K in the soil solution, as the SL soil used in this study had pH range of 6.5–7.8 (Sachs, 2004).

The exchangeable Ca content was significantly higher than the Control only for the PL400 treatment in the SL soil, while no biochars had an effect on Ca content in the SY soil despite the high input with all biochars. The solubility of Ca and consequently its availability is mainly determined by the soil pH and soil P content. The low exchangeable Ca content of manure biochar treated SY soil is explained by the binding of Ca with biochar P resulting into the formation of insoluble calcium phosphates as the total P supplied through manure biochar into the soil was significant (up to 575 mg P kg^{-1} with PL600 treatment) (Cui et al., 2011; Wang et al., 2015a). Since the SY soil used in this experiment was highly calcareous (15% $CaCO_3$), significant amount of Ca is unexchangeable as this could have been precipitated as insoluble $CaCO_3$ (Wojtowicz, 1998). Furthermore, we observed that the low exchangeable Ca in SY soil is also regulated by the soil pH as the Ca availability starts decreasing slowly pH above 8.5 and biochar treated SY soil in this study ranged between 8.5–9.2 (Wojtowicz, 1998; Sachs, 2004).

Insoluble phosphate salts could subsequently be made available for the crops through the phosphate mobilizing bacteria or root exudation of organic acids (Gyaneshwar, 2002).

The result of the MBC in this experiment seems to be completely different in the two soils. In the SY soil, all biochars significantly increased MBC compared with the Control. The mechanisms that might have resulted in the increased MBC from the biochar amendments in this soil include metabolism of labile organic C compounds in the biochars (Bruun et al., 2008; Smith et al., 2010; Bruun et al., 2011), microbial access to nutrients on biochar surfaces (Cheng et al., 2008), enhanced microbial activity due to rapid decomposition of SOM (Wardle et al., 2008), and physical protection for microbes as biochar pores and surfaces could serve as habitat for the microbes (Lehmann et al., 2011). In contrast, the MBC values for PL400 and WC treatments did not differ significantly compared to the Control in the SL soil. A possible explanation could be that the addition of labile C through the biochars might have led to no noticeable significant effect on the MBC as the SL soil was characterized as rich in OM. Surprisingly, the MBC values were significantly lower for the PL600, SM400 and SM600 compared with the Control in the SL soil. It is possible that a mechanism other than, or in addition to, soil organic C content may have been responsible for the significantly lower values of MBC exhibited by these biochar amended soil. This is beyond the scope of this research. Since significant plant growth promotion was recorded under the SM400 (compared with the Control) and PL600 (compared with the WC) treatments, the role of soil microbial biomass could have been less important than direct nutrient supply in respective biochar treatments. Therefore, our results of MBC beg further experimental analytical verification.

The dehydrogenase is an intracellular enzyme and is likely not to be affected much by the quorum sensing, the so called production and release of the chemical signal molecules (Masiello et al., 2013). The readily available C components (e.g. simple soluble sugars, volatile organic compounds) of the low temperature manure biochars (PL400 and SM400) could stimulate the heterotropic microbial activity and subsequently increase the DA (Van Zwieten et al., 2013, Ameloot et al., 2014, 2015). As the DA was positively correlated with VM content and negatively correlated with C:N ratios of the biochars in both soils, this supports the idea of enhanced microbial consumption of volatile C triggering such enzyme activity as suggested by Ameloot et al. (2015). The lower DA with WC biochar treatment (in both soils) and with PL600, SM600 treatments (in the SY soil) compared to the Control could be explained by substrate blocking or adsorption into high temperature biochar surfaces (Ameloot et al., 2014; Bailey et al., 2011). In addition, it also indicates that the high temperature biochars are more recalcitrant as well as resistant against microbial attack, showing the potential of these biochars in sequestering C in the long-run (Subedi et al., 2016). The correlation between soil pH and DA was also significantly positive in the SL soil, while this was not in the SY soil though positive, suggesting that the liming potential of manure biochars is of importance in stimulating DA in the SL soil (Ameloot et al., 2015).

The β-glucosidase is an extracellular enzyme and is likely to be affected by the quorum sensing chemicals (Masiello et al., 2013). Increased activity of this enzyme fosters further microbial activity when initial pools of labile C compounds (e.g. glucose) are consumed rapidly by the microbes, thus targeting on high molecular weight C compounds (e.g. lignin and cellulose) mainly due to the change in substrate use patterns (co-occurrence) of soil microorganisms as a result of

biochar additions (Eivasi and Tabatabai, 1988, Lehmann et al., 2011). The stimulation of such enzyme is considerably affected by the accumulation of labile organic compounds resulting in its catabolic repression due to high level presence of glucose (Ameloot et al., 2014; Kotroczó et al., 2014). The negative correlation between GA and the biochar VM contents would support this hypothesis on stimulation of such enzymes.

The higher level of GA for the Control treatments in both soils can be explained by the presence of high molecular weight organic compounds (e.g. polymers) in the native SOM, showing a dominant role of GA over DA in breaking down the cellulosic C into simple sugars such as glucose. The correlation between GA and soil pH was also negative in both soils. Moreover, an increase in DA in both soils was accompanied by the decrease in GA and vice versa. This was supported by the negative correlation between DA and GA in both soils, suggesting that these two enzymes act differently based on substrate availability, their sorption and desorption into biochar surfaces and their differing response is considerably affected by the presence of labile organic matter in the soil biochar mixture (Spokas et al., 2011; Kotroczó et al., 2014; Ameloot et al., 2015). Additionally, a significant positive correlation between GA and biochar C:N ratios further suggests that lacking labile C substrate reduces DA activity, but increases GA as an alternative means of providing C as food for the microbes (Awad et al., 2012; Ameloot et al., 2014; Wang et al., 2015b). In general, these two enzymes provide insights on the degree of resistance of organic matter against microbial degradation in soils amended with biochars. They show that labile C availability is higher in manure-derived biochars *vs* standard wood-derived biochar, in poultry manure biochars *vs* swine manure biochars, in low *vs* high pyrolysis temperature (Lehmann et al., 2011; Award et al., 2012).

While only some biochar applications had a significant effect onto plant growth, soil nutrient status, soil enzymatic activities and nutrient uptake, all soil bacterial communities were significantly affected by the biochar deposition as revealed by the CCA and permutation analysis, including WC. In contrast, pyrolysis temperature had a limited impact on the bacterial communities as only PL400 and PL600 could be significantly separated in SY. Significant changes in bacterial community structures may result into beneficial effects to soil nutrient availabilities, when key nutrient mobilizing bacteria increase in abundance or activity as for instance identified by Fox et al. (2014). There, higher abundances of both S- and P-solubilizing bacteria were found in biochar treated soil of low P availability alongside significant contributions of such bacteria in shifting the community structure. Likewise, aromatic sulfonates present on the manure biochars could also have been desulfurized by desulfonating bacteria as Schmalenberger and Noll (2014) found a significant response of these bacteria to aromatic sulfonate addition in grassland soils. Future investigations in phosphatase and sulfatase activities on enzymatic and gene expression levels may close this remaining knowledge gap (Schmalenberger and Fox, 2016). In this study, shifts in bacterial community structures were significantly correlated with changes in soil pH, soil C, TN, C/N ratio and plant growth. Physico-chemical changes in the amended soils including pH may explain some but not all of the observed community shifts (Jones, 2009; Lehmann et al., 2011) as no pH change in WC amendment was detected in both soils. In SL, effects of significantly high shoot DM (in PL400 and SM400) were not reflected in significantly altered bacterial community structures. However, differences were found in SY, where significant increases in shoot and root DM (in PL400) were correlated to a shift in the bacterial community structure. These findings suggest that the altered

bacterial communities of PL400 amendments in SY may have contributed to increased nutrient mobilization and plant growth after all.

Although this study excludes non-pyrolyzed manure (raw feedstock) as a reference treatment for comparison, previous study reported that pyrolysis of manure (poultry litter) resulted a biochar with higher C stability associated with greater aromaticity as well as higher P and K fertilizer value than the raw feedstock, potentially adding both agronomic and environmental benefits (Van Zwieten et al., 2013). In addition to these benefits, we recommend further investigation of the economic potential of this pyrolysis technique compared with other technologies, such as composting and anaerobic digestion of manure feedstock in agricultural sector considering a life cycle analysis of the entire system.

5. Conclusions

The total DM yields of ryegrass (both above and belowground biomass) were related to both positive nutrients content (NPK and cations) of biochars and enhanced soil characteristics (chemical and biological). This shows that low temperature manure biochars (PL400 and SM400) can be utilized as potential NPK-fertilizers with a significant value as most of the nutrients can be recovered into the char. None of the biochars were found to be toxic for the ryegrass growth as they all boosted the growth, despite being high in VM content. All biochars significantly increased the soil C contents compared to the Control after ryegrass harvest, with WC biochar showing the greatest C sequestration potential. All manure biochars showed a positive liming effect in acidic silt-loam soil, and increased nutrient availability (except Ca) in both soils.

Despite manure biochars showed positive enhancement in relation to yield and soil fertility (both chemical and biological), they were not successful in compensating differences between soil type. The more fertile soil enhanced the possibility to show differences among biochar types. As this work mainly focussed on assessing the fertilizing value of manure biochars at a small scale lab experiment, field trials with careful considerations of environmental issues (e.g. eutrophication, leaching) due to potential excessive nutrient load (mainly P and Ca) are required before applying such materials in the field for making specific agronomic recommendation. In addition, further investigations looking into the technical and economic viability of manure pyrolysis in relation to nutrient flow and recovery, plus a feasibility study compared to other manure management practices, such as composting or anaerobic digestion, are also recommended.

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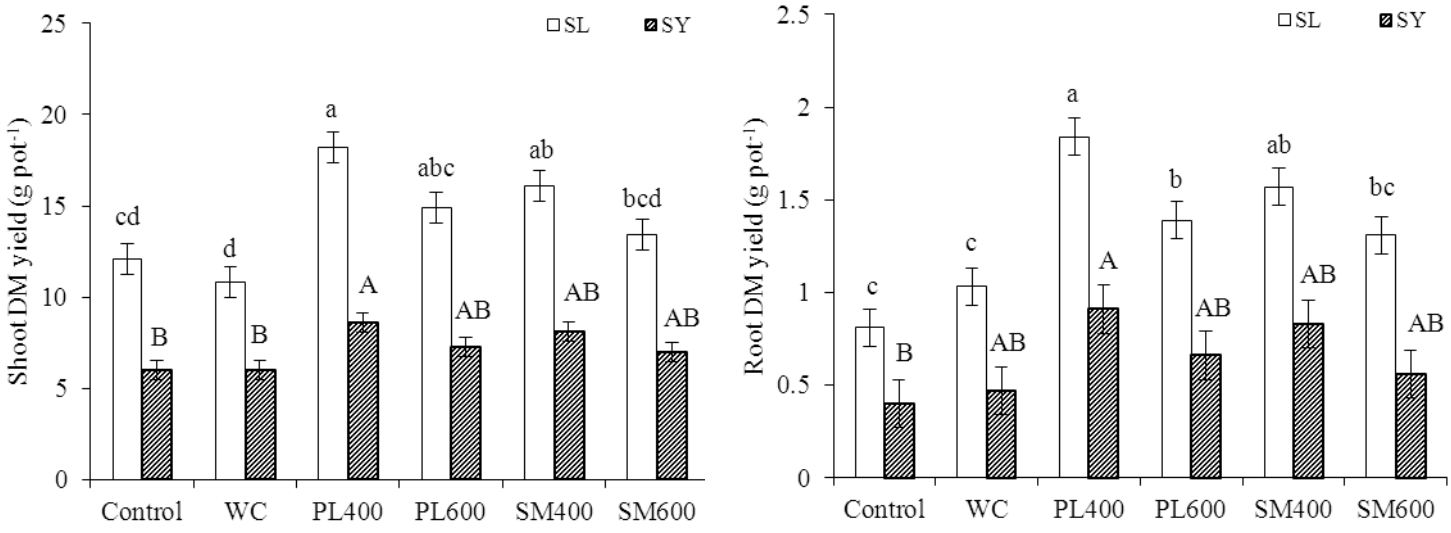

Fig. 1. Effects of biochar amendment on above (graph on the left) and belowground (graph on the right) biomass yield. Error bars represent standard error of the means (n=3). Please note different scales of Y-axis. Different small letters indicate significant differences (p<0.05) in SL soil (empty bars), while capital letters indicate differences (p<0.05) in SY soil (striped bars) between different treatments for shoot and root DM yield.

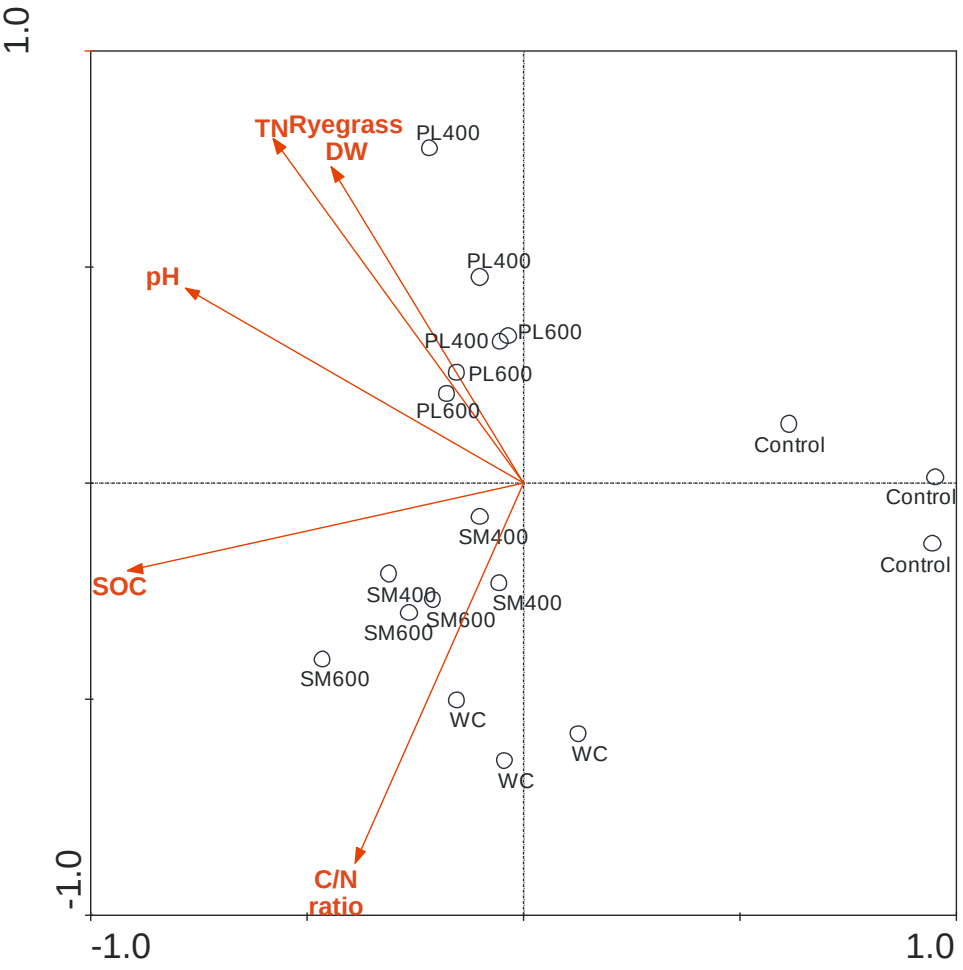

Fig. 2a. CCA plot showing the effects of biochar amendments on 16S rRNA gene based bacterial community structure in soil-loam (SL) soil (n = 3) with soil pH, SOC, TN, C/N ratio and ryegrass yield (DW) defined as environmental factors. Arrows for each variable tested indicate significance ($p \leq 0.05$, permutation test) of environmental factors on shift of the bacterial community structure upon biochar amendment.

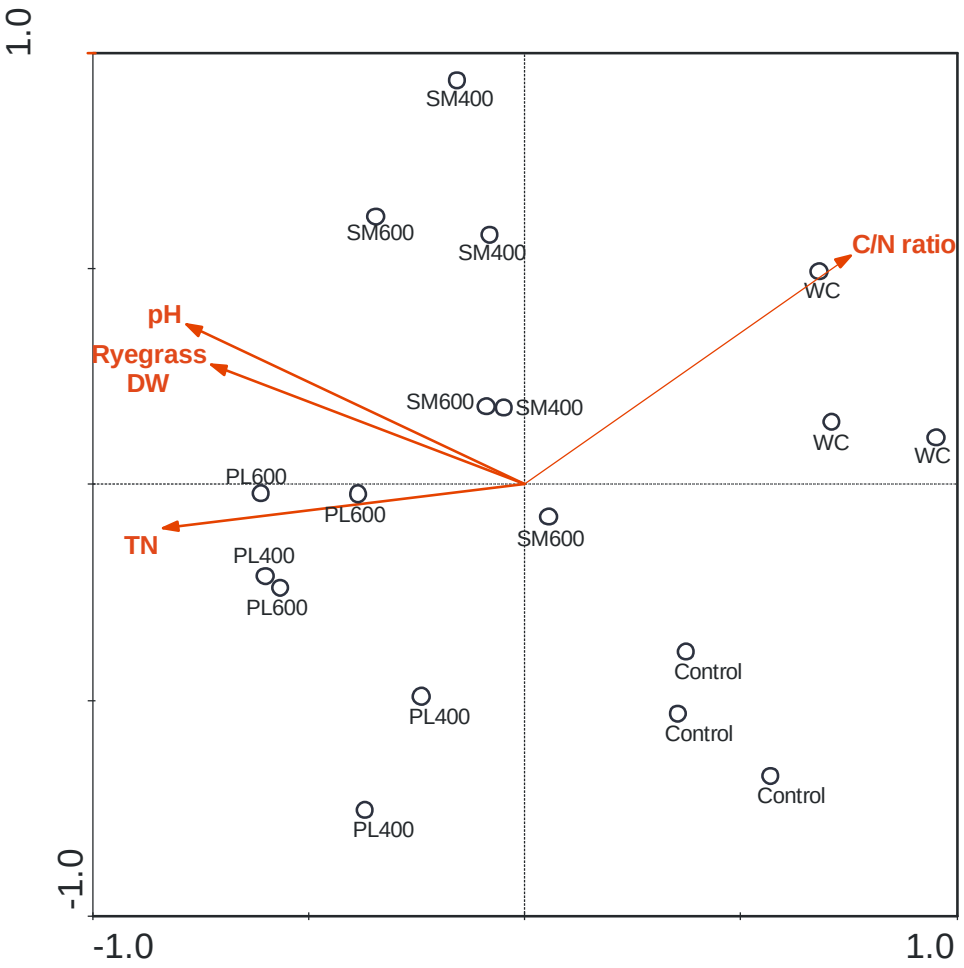

Fig. 2b. CCA plot showing the effects of biochar amendments on 16S rRNA gene based bacterial community structure in sandy (SY) soil (n = 3) with soil pH, TN, C/N ratio and ryegrass yield (DW) defined as environmental factors. Arrows for each variable tested indicate significance ($p \leq 0.05$, permutation test) of environmental factors on shift of the bacterial community structure upon biochar amendment.

Table 1

Properties of the soils used in the experiment.

Soil type	Sand	Silt	Clay	Porosity[a]	$CaCO_3$	SOC	TN	C/N	pH	CEC	P[b]	K[c]	Ca[c]	Mg[c]
	(%)							–	–	($cmol_c$ kg^{-1})	(mg kg^{-1})			
SL	17.2	71.1	11.7	49.2	0.4	1.2	0.15	8.0	6.1	12.4	23	42	1452	179
SY	89.7	5.5	4.8	45.3	15.3	0.52	0.057	9.1	8.3	5.4	14	28	980	21

Abbreviations: SL = silt-loam, SY = sandy, SOC = soil organic carbon, TN = total nitrogen, CEC = cation exchange capacity.

[a] calculated on the basis of bulk density and particle density.

[b] available (Olsen P).

[c] exchangeable.

Table 2

Physico-chemical characteristics of different biochars utilized in the experiment.

Biochar type[a]	TC (%)	TN	VM	Ash	C:N –	CEC ($cmol_c\ kg^{-1}$)	P[c] ($g\ kg^{-1}$)	P[b]	Ca[c]	Mg[c]	K[c]	pH –	Fe ($mg\ kg^{-1}$)	Al	Zn	Cu	Mn	Ni	Pb	SA ($m^2\ g^{-1}$)	PV ($cm^3\ g^{-1}$)
PL400	52.1	5.85	44.9	25.3	9.0	30.2	20.0	12.3	28.3	17.3	38.8	9.5	2909	537	1164	349	1099	52	13	5.4	0.006
PL600	52.8	4.01	24.7	35.4	13.0	27.5	28.7	15.4	35.9	24.0	58.8	10.4	4311	777	1633	366	1437	52	13	6.3	0.012
SM400	54.9	2.23	29.9	27.5	24.6	52.5	22.1	9.7	20.3	15.7	16.2	10.0	5392	617	585	156	455	26	bdl	5.8	0.009
SM600	57.9	1.79	17.8	34.5	32.4	18.6	28.2	15.6	28.9	21.3	35.3	10.4	6674	834	770	180	513	26	13	10.6	0.01
WC	89.3	0.27	15.3	7.8	335.4	14.8	0.92	0.7	13.6	3.2	2.6	11.0	1322	1097	79	53	397	40	13	178.3	0.14

Abbreviations: Same as given in Table 1 as well as: TC = total carbon, VM = volatile matter, SA = surface area, PV = pore volume, bdl = below detection limit.

[a] Letters refer to feedstock material as poultry litter (PL), swine manure (SM) and wood chip (WC), numbers refer to pyrolysis temperature in ºC, with addition to WC at 1000 ºC.

[b] Available.

[c] Total.

Table 3

Effects of biochar amendments on above ground nutrient uptake and uptake efficiency on two soils (n = 3).

	Above ground uptake (mg pot^{-1})			Uptake efficiency[a] (%)		
Treatment	N	P	K	N	P	K
			SL soil			
Control	478.1 c	47.3 c	304.1 d			
WC	457.9 c	37.6 c	369.1 cd	-24.8[b]	-44.7	83.4
PL400	785.9 a	123.7 a	943.2 a	17.5	20.8	54.9
PL600	511.2 bc	96.5 ab	807.1 a	2.7	10.6	28.5
SM400	671.6 ab	103.6 ab	629.1 b	28.9	19.3	66.9
SM600	460.2 c	87.3 b	451.2 c	-3.3	8.6	13.9
SE	46.6	7.33	37.1			
p	0.001	<0.001	<0.001			
			SY soil			
Control	209.9 b	25.1 b	218.1 d			
WC	204.8 b	22.5 b	262.5 cd	-6.3	-11.6	57.0
PL400	291.8 a	49.8 a	527.3 a	4.7	6.7	26.6
PL600	287.5 a	36.5 ab	484.4 a	6.5	2.5	15.1
SM400	285.1 a	44.4 a	337.5 bc	11.2	6.7	24.6
SM600	227.1 ab	33.8 ab	303.4 cd	3.2	1.9	8.1
SE	15.4	4.32	29.8			
p	0.003	0.005	<0.001			

Abbreviations: Same as given in Tables 1 and 2 as well as: SE = standard error of the mean.

Mean values followed by the same letter are not significantly different ($p<0.05$).

[a] (Treatment – Control)/NPK supplied via biochar * 100, respectively for N, P and K uptake.

[b] Calculated based on average uptake values for each treatment per soil type.

Table 4

Effects of biochar amendments on chemical and microbial properties of silt-loam (SL) and sandy (SY) soils (n = 3) at the end of the ryegrass growth experiment.

Treatment	C[a]	TN	pH	P[b]	Ca[c]	Mg[c]	K[c]	CEC	MBC	DA[d]	GA[e]
	(%)		–	(mg kg^{-1})				($cmol_c$ kg^{-1})	(µg g^{-1})	(µg g^{-1} h^{-1})	
						SL soil					
Control	1.29 b	0.13 c	6.56 c	20.8 e	1890.1 b	246.9 d	10.8 c	16.1	186.6 a	48.6 c	187.7 ab
WC	2.74 a	0.14 c	6.81 c	20.8 e	2010.2 b	253.5 d	24.7 c	16.3	167.2 ab	18.4 d	215.1 a
PL400	2.12 a	0.23 a	7.84 a	98.2 b	2347.1 a	281.2 c	37.8 bc	16.5	189.2 a	89.1 a	127.1 cd
PL600	2.29 a	0.21 ab	7.88 a	89.2 c	1890.4 b	311.3 b	119.4 a	14.6	109.1 bc	56.8 bc	149.5 bc
SM400	2.45 a	0.18 abc	7.36 b	77.1 d	1899.4 b	331.9 b	57.5 b	16.4	45.1 c	92.2 a	92.2 d
SM600	2.43 a	0.17 bc	7.88 a	106.4 a	1999.8 b	372.1 a	22.2 c	16.0	58.5 c	80.1 ab	152.1 bc
SE	0.14	0.01	0.10	1.35	68.3	5.46	6.14	0.90	15.6	6.51	9.21
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.00	0.710	<0.001	<0.001	0.001
						SY soil					
Control	0.91 c	0.06 c	8.59 c	12.6 d	1042.1	84.6 c	18.1 d	6.93	67.6 c	48.4 b	288.2 a
WC	2.72 a	0.08 bc	8.64 c	14.2 d	1027.5	87.9 c	9.4 d	6.49	150.7 ab	4.88 d	310.7 a
PL400	1.66 b	0.15 a	8.83 b	108.1 b	862.3	149.4 b	115.1 b	6.89	185.3 a	59.3 a	190.8 b
PL600	1.89 b	0.14 a	9.21 a	136.5 a	885.8	182.7 a	143.8 a	6.76	124.6 b	32.1 c	177.6 b
SM400	1.97 b	0.09 bc	8.99 b	129.3 a	957.9	154.1 b	82.7 c	7.38	114.8 b	46.7 b	187.3 b
SM600	1.75 b	0.11 ab	8.97 b	83.7 c	1018.9	136.2 b	88.1 c	7.78	175.2 a	27.2 c	292.7 a
SE	0.14	0.01	0.03	2.21	46.7	3.97	5.88	0.52	4.43	2.1	16.8
p	<0.001	0.001	<0.001	<0.001	0.072	<0.001	<0.001	0.533	<0.001	0.001	<0.001

Abbreviations: Same as given in Tables 1 and 3 as well as: SE = standard error of the mean, MBC = microbial biomass carbon, DA = dehydrogenase activity, GA = β-glucosidase activity.

Means followed by the same letter are not significantly different ($p<0.05$).

[a] After subtracting inorganic C from the total soil C.

[b] Available (Olsen).

[c] exchangeable.

[d] Expressed as produced level of iodonitrotetrazolium formazan (INTF), and

[e] As produced level of p-nitrophenol (PNP).

Chemically and biologically-mediated fertilizing value of manure-derived biochar

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HIGHLIGHTS

- Low temperature manure-derived biochars enhanced both crop yield and NPK uptake, and improved soil properties.
- Manure biochars showed more positive effects on acidic silt-loam soil than on alkaline sandy soil.
- Wood chip biochar had no effect on crop yield, but showed a good C sequestration potential.
- All biochars shifted bacterial community structure and modified enzyme activities.

Key words: Pyrolysis temperature, amendment, fertilizer, crop growth, carbon sequestration, soil enzyme activity

ABSTRACT

This study evaluates the potential of manure-derived biochars in promoting plant growth and enhancing soil chemical and biological properties during a 150 day pot experiment. Biochars from pyrolysis of poultry litter (PL) and swine manure (SM) at 400 and 600°C, and a commonly available wood chip (WC) biochar produced at high temperature (1000 °C) were incorporated to silt-loam (SL) and sandy (SY) soils on a 2% dry soil weight basis. Ryegrass was sown and moisture was adjusted to 60% water filled pore space (WFPS). The PL400 and SM400 biochars significantly increased (p<0.05) shoot dry matter (DM) yields (SL soil) and enhanced nitrogen (N), phosphorus (P) and potassium (K) uptake by the plants in both soils, compared to the Control. All biochars significantly increased the soil carbon (C) contents compared to the Control. Total N contents were significantly greater for PL400 and PL600 treatments in both soils. The dehydrogenase activity (DA) significantly increased for PL400 and SM400 treatments and was positively correlated with the volatile matter (VM) contents of the biochars, while β-glucosidase activity (GA) decreased for the same treatments in both soils. All biochars significantly shifted (p≤0.05) the bacterial community structure compared to the Control. This study suggests that pyrolysis of animal manures can produce a biochar that acts as both soil amendment and an organic fertilizer as proven by increased NPK uptake, positive liming effect and high soil nutrient availability, while WC biochar could work only in combination with fertilizers (organic as well as mineral).

Introduction

Biochar, a carbonaceous solid recalcitrant pyrogenic material, has drawn considerable scientific attention due to its potential in climate change mitigation, waste management, soil fertility enhancement and crop growth promotion (Chan et al., 2007; Hossain et al., 2011; Ameloot et al., 2014; De La Rosa et al., 2014; Jeffery et al., 2015). The environmental benefits of biochar application to soils are associated with increased carbon (C) sequestration (Lin et al., 2015) and suppression of greenhouse gas (GHG) emissions (Kammann et al., 2012), while agronomic benefits include improved soil structure and porosity, increased surface areas, water holding and cation exchange capacities (CEC) of biochar amended soils (Lehmann et al., 2003; DeLuca et al., 2009; Cornelissen et al., 2013). However, there still remains some dispute regarding biochar use in soils as few reports comment on their negative effects on to crop growth (Deenik et al., 2010; Wisnubroto et al., 2011), due to toxic volatile compounds that are potentially formed during pyrolysis (Spokas et al., 2011), and these could also eventually affect the soil microbiota (Gul et al., 2015).

The biochar-amended soil nutrients (both micro and macro) availability depends on many physico-chemical char properties, such as pH, surface area (SA), porosity, CEC and the transfer of nutrients into the amended soil (DeLuca et al., 2009; Clough et al., 2013). In addition, manure biochars also have potential as liming agent for acid soils because of their high ash contents (Srinivasan et al., 2015; Subedi et al., 2016). The crop growth promotion in biochar amended soils is thought to be enhanced nutrient use efficiency in addition to reduced leaching associated with positive enhancement of soil chemical (Lehmann et al., 2003; Knowles et al., 2011; De La

Rosa et al., 2014), and microbial properties (Nielsen et al., 2014, Gul et al., 2015). Low nutrient content biochars produced mainly from wood biomasses, also known as charcoal, show positive effects on crop growth only when applied in combination with fertilizers (Steiner et al., 2007, Deenik et al., 2010; Subedi et al., 2015; Schmidt et al., 2015). In contrast, manure biochars may also act as biofertilizers as their original feedstocks are rich in nutrients (Singh et al., 2010; Hossain et al., 2011; Cantrell et al., 2012), with limited information on their agronomic value and thus seeking for further experimental evidence.

The stability of biochar in soils depends on several concurrent factors such as feedstock quality, type of thermal process (low/high temperature pyrolysis, slow/fast pyrolysis, gasification, hydrothermal carbonization), the processing conditions (temperature and residence time), the resulting biochar properties as well as the environmental factors (Zimmermann et al., 2011; Kammann et al., 2012; Ameloot et al., 2014; Gul et al., 2015; Subedi et al., 2016). The recalcitrant biochar carbon and its aromatic structure might interfere with the natural environment of soil organic matter and affect microbial diversity, abundance and community composition (Lehmann et al., 2011). The highly porous structure of biochar can provide protection and an aerated habitat for the mycorrhizal fungi and bacteria, potentially changing the bacterial biogeochemical nitrogen (N), phosphorus (P) and sulphur (S) cycles, and their community composition (O'Neill et al., 2009; Fox et al., 2014; Schmalenberger and Noll et al., 2014). These changes are among the drivers for plant growth promotion based on evidences of nitrification, N-fixation, P- and S- mobilization by the respective functional bacteria (Lehmann et al., 2011; Fox et al., 2014).

Biochar's effect on soil microbial biomass has been widely reported (Lehmann et al., 2011; Gul et al., 2015). As biochar can modify soil properties such as soil structure, pH, CEC and the availability of organic C, it will influence the size of the microbial biomass (Cheng et al., 2008; Smith et al., 2010; Lehmann et al., 2011; Bruun et al., 2011). The amount of labile C from the biochar is of particular importance as this provides a readily available carbon energy source that increases microbial decomposition and finally microbial biomass (Gonzalez-Quiñones et al., 2011). Biochar can also have a major effect on soil microbial enzymatic activity (Ameloot et al., 2014, 2015). Enzymes such as dehydrogenase (intracellular) and β-glucosidase (extracellular) represent the indicators of soil microbial activity in assessing the degree of resistivity of organic matter (OM) in biochar-amended soils against microbial degradation. The activities of these enzymes are thus influenced by the nature of C compounds (labile and/or aromatic) present in the biochar (Camina et al., 1997), the pyrolysis temperature, the nature of the enzyme itself, and the incubation time (Wang et al., 2015b).

Manure may not be an ideal feedstock for thermal treatment, such as combustion and gasification at high temperature, due to its high moisture and alkali metal content, causing ash agglomeration (Di Gregorio et al., 2014; Lynch et al., 2013). Pyrolysis operates at lower temperatures (300–600 °C) compared to combustion and gasification, reducing the risk of ash agglomeration and harmful emissions during combustion (NO_x, SO_2, particulates) (Basu, 2010). Nevertheless, from a waste management perspective there are good reasons why manure should be addressed to thermal treatment, particularly as recent EU legislations are trying to enforce a more sustainable agricultural system. The main advantages are a reduction of waste in volume

and mass, reduction of pathogens and odour compounds, reduced nutrient runoff and added income from selling ash, char and gas (Arena, 2012).

The potential agronomic and environmental benefits of producing biochar from manure, and the possibility of using it as a soil amendment has been less explored compared with the commonly available biochars produced from wood biomasses, and is of scientific interest in the manure management chain (Sing et al., 2010; Cantrell et al., 2012; Jensen, 2013b). Moreover pyrolysis could be an option to stabilize OM in the manure feedstock and recover most of the nutrients in the mineral ash fraction, despite significant cost of this process and the N and S losses are unavoidable (Cantrell et al., 2012; Jensen, 2013a; Huang et al., 2015).

A previous experiment demonstrated high microbial respiration in soil, high N mineralization potential and high availability of N, P, K and other cations as well as the liming potential of manure-derived biochars (Subedi et al., 2016). We opted to further explore these findings by showing that manure-derived biochars have the potential to sustain crop growth due to induced physical, chemical and biological changes in soil. We therefore continued the previous experiment using the same experimental substrates under controlled environmental conditions, with the following hypotheses: (i) low temperature pyrolysis of manure feedstock produces biochar with a high fertilizing value and low toxicity, (ii) high temperature pyrolysis is effective in producing a recalcitrant amendment, and (iii) pyrolysis conditions (temperature and feedstock) modulate the effects on residual soil chemical and microbial properties.

2. Materials and methods

2.1. Soil collection and characterization

The two soils used in this experiment had contrasting characteristics (Table 1): i) a silt-loam soil, referred as "SL", high in OM with slightly-acidic pH, and ii) a sandy soil, designated as "SY", low in OM with moderately alkaline pH (Subedi et al., 2016). The two soils were taxonomically classified as Alfisol (SL soil) and Entisol (SY soil) according to the USDA soil classification system (USDA, 1999). Both soils were low in N, P and K contents, but rich in Ca contents. They were both collected from the top 20 cm plough layer of arable fields (NW Italy). The soils were then air-dried and mechanically sieved (2 mm particle size), using an electric auto-rotating sieving device (Neotron s.r.l., Autopack, Modena, Italy). Soil texture was determined by the pipette method (Gee and Bauder, 1994), soil bulk density by the core method and particle density by the pycnometer method (Blake and Hartge, 1994). Total pore volume was calculated based on the bulk density and particle density values obtained. The soils were analysed for total C and N contents using a total elemental analyser (Vario El Cube, Elementar, Hanau, Germany). The chemical analysis (pH, Olsen P, K, Ca, Mg and CEC) was carried out following routine analytical procedures (Sparks, 1996).

2.2. Biochar production and characterization

The biochars used for this experimental study were produced from two different manure feedstocks (poultry litter (PL) and swine manure (SM)) at two different pyrolysis temperatures (400 and 600 °C) (Table 2). The poultry litter biochars ("PL400" and "PL600") were produced at

the University of Limerick (Ireland), using a laboratory pyrolysis plant, while the swine manure biochars ("SM400" and "SM600") were supplied by ECN in the Netherlands (www.ecn.nl). Another widely available commercial biochar from wood chip (WC) was included as a reference material. This was produced from kiwi tree pruning residue via industrial gasification (1000 °C) at Agrindustria, Italy (www.agrind.it). A biochar-free control completed the experiment.

Biochars were physically characterized for surface area (SA) and pore volume (PV) according to the Brunauer, Emmet and Teller (BET) method, via the measurements obtained by N_2 adsorption at 77K using an ASAP-2400 Micrometrics apparatus (Table 2). They were chemically characterized for total C, N, available P (2% formic acid extractable), cations (K, Ca and Mg), pH and CEC as mentioned in Subedi et al. (2016). The total P and heavy metal contents were analysed by Atomic Absorption Spectroscopy (Varian Techtron AA6, Melbourne, Australia), following acid digestion of biochar samples (Cantrell et al., 2012). Proximate analysis was carried out for the determination of the ash and volatile matter (VM) contents according to the NSAI standard testing method (NSAI, 2009). Each measurement was carried out in triplicate. As reported by Subedi et al. (2016), with respect to WC biochar, all manure biochars were higher in nutrient contents, VM and CEC, lower in C contents, surface areas and pore volumes (Table 2). Except for Al and Pb, the heavy metal concentrations for the manure biochars were also higher compared to the WC biochar.

2.3. Plant growth experiment setup

The growth experiment was organized in a completely randomized design with three replicates in a controlled climatic chamber (20 ºC, 65% relative humidity and photon flux of 260 µmol m^{-2} s^{-1}). The soil used in this experiment derived from the previous one started 9 months before, when the two soils were air dried, sieved (2 mm), amended with biochar (2% w/w), rewetted and fertilized with NH_4NO_3 (>99% purity, Merck KGaA, Darmstadt, Germany) at 170 kg N ha^{-1}, as fully described in Subedi et al. (2016).

After pooling the replicates from that experiment, amended soils were repacked into plastic pots (1.5 kg for each replicate, d=13.5, h=13.5 cm), and moistened to 60% water filled pore space (WFPS) using normal irrigation water (<6 mg l^{-1} NO_3^--N, negligible PO_4^{3-}). Soils were allowed to stabilize for a week then, seeds of Italian ryegrass (*Lolium multiflorum L.*) were sown (0.2 g pot^{-1} given the seed rate of 10 g m^{-2}) on the soil surface (0.0182 m^2) and gently pressed with finger to ensure maximum soil-seed contact. The soil water content was adjusted every three days by weighing the pots and adding water to achieve the original moisture content. No extra fertilization was undertaken after sowing. A total of five harvests were completed during the entire growth period of 150 days and dry matter (DM) yield was recorded.

2.4. Plant and soil analysis

Each of the five harvests was performed by cutting the above ground biomass approximately 2 cm above the soil surface, dried at 40 °C for 72 h and dry weights were recorded. After completion of the growth experiment, roots were carefully hand separated from the soils, thoroughly washed with water and dried using the same procedure as for the shoots. Total shoot

N content was determined by a total elemental analyser (Vario El Cube, Elementar, Hanau, Germany). Total shoot P and K contents were analysed by calcination followed by acid recovery using inductive coupled plasma mass spectrometry (iCAP^{TM} Q ICP-MS, Thermo Scientific^{TM}, Pittsburgh, USA). The NPK uptakes were calculated as a product of tissue NPK concentration and biomass yield. The biochar NPK uptake efficiencies (UEs) were also calculated from the increase in uptake between the Control and biochar treatments (Equation 1), and expressed as percentage of NPK supplied from the biochars as described by Jensen (2013b).

$$UE = \frac{Uptake\ (treat.) - Uptake\ (ctrl.)}{NPK\ supplied\ via\ biochar} \times 100\% \quad (1)$$

Where,

UE = 'N' or 'P' or 'K' uptake efficiency (%),

Uptake (treat.) = NPK uptake (mg pot^{-1}) with biochar treatments,

Uptake (ctrl.) = NPK uptake (mg pot^{-1}) with the Control treatment, and

NPK supplied = Total N or P or K (mg pot^{-1}) supplied to the soil from the respective biochars.

At the end of the growth experiment, the biochar-amended soils were characterized both chemically and biologically. The soils were analysed for total C and N contents using a total elemental analyser (Vario El Cube, Elementar, Hanau, Germany), while pH, available P (Olsen) and CEC following routine analytical procedures (Sparks, 1996). The soil mineral N was analysed colorimetrically following the procedure in Subedi et al. (2016). Soil microbial biomass carbon (MBC) was determined by the fumigation-extraction method (Beck et al., 1997). The dehydrogenase activity (DA) was determined following a procedure described in Camina et al.

(1997). The protocol for β-glucosidase activity (GA) was adapted from Eivasi and Tabatabai (1998). Soils were stored at 4 ºC for MBC and enzyme determination and at -25 ºC for DNA extraction until analysis.

2.5. Soil DNA extraction and measurement, and bacterial community structure analysis

Soil DNA was extracted from the bulk soil following a protocol of the POWER SOIL® DNA isolation kit (MO BIO Laboratories, Cupertino, CA, USA) as per guidelines from the manufacturer. The extracted DNA was quantified spectrophotometrically (NanoDrop ND 1000, Thermo Scientific, Pittsburgh, USA). The 16S rRNA gene from 5-10 ng template DNA was subsequently amplified using the universal primers 348GC and 518R (Muyzer, 1993) with a touch-down polymerase chain reaction (PCR) protocol as outlined previously (Fox et al., 2014). Subsequent soil bacterial community structure analysis was carried out via denaturing gradient gel electrophoresis (DGGE) at a gel strength of 10 % with a 35 to 65 % denaturing gradient and electrophoresis was conducted for 1040 Vh at 60°C in TAE buffer (Fox et al., 2014). Gels were stained after electrophoresis with SybrGold (Invitrogen, Carlsbad, CA) and DNA bands were visualized in a UV transilluminator (G:Box, Syngene, Cambridge, UK). Phoretix 1D was used to analyse the DGGE profiles and to create a binary matrix (Totallab, Newcastle upon Tyne, UK).

2.6. Data processing and analysis

All data concerning ryegrass yield, nutrient uptake and soil properties were analysed using a one-way ANOVA (IBM SPSS statistics 22) separately for each soil type. Statistical significance

was tested at $p<0.05$. The validity of model assumption for each variable was verified by examining the residuals for normal distribution (Shapiro-Wilk test) and homoscedasticity (Levene's test). Data violating the model assumptions were logarithmically transformed, analysed by ANOVA and the back transformed values to the original scale were reported. A common standard error (SE) of the mean from the pooled variance, for each measured variable, was reported for all the treatments. Tukey's HSD post-hoc test was applied for pairwise comparison to assess any significant differences ($p<0.05$) between treatment means. Additionally, a Pearson bivariate correlation analysis was performed for the different measured variables to assess any correlation of the biochar properties with ryegrass yield, NPK uptake, and soil microbial properties.

Binary matrices from 16S rRNA gene fragment based DGGE were analysed via canonical corresponding analysis (CCA) alongside environmental factors (e.g. soil pH, SOC, TN, C/N ratio and ryegrass yield) using CANOCO (Microcomputer Power Inc., Ithaca, NY). Permutation tests were conducted (9,999 repeats) to identify differences of DGGE profiles at significance level ($p\leq0.05$) as described by Noll (2008).

3. Results

3.1. Ryegrass yield, nutrient uptake and efficiency

Results from ryegrass growth demonstrated that the low temperature manure-derived biochars (both PL400 and SM400) significantly increased both shoot and root DM yield compared to the

Control in the SL soil, while PL600, SM600 and WC biochars had no effect on either shoot or root DM production (Fig. 1). Only the PL400 treatment significantly increased shoot and root DM in SY soil. The highest shoot DM yield increase of 50.1% compared to the Control was recorded in PL400 treated SL soil, followed by 44.0% with PL400 treated SY soil. Similarly, up to 127.2% increase of root biomass (DM) compared to the Control was observed for PL400 treated SL soil followed by 93.8% increase with SM400 treatment. The biochar N content was significantly positively correlated with both the shoot DM yield (n=15, $p<0.01$, r=0.78 for SL and $p<0.05$, r=0.61 for SY) and the root DM yield (n=15, $p<0.01$, r=0.82 for SL and $p<0.05$, r=0.59 for SY).

The N uptake significantly increased compared to the Control and WC for the PL400 and SM400 treatments in the SL soil, while this was significantly increased for all manure biochar treatments in the SY soil (Table 3). The highest increase in N uptake by 64.4% compared with the Control was recorded in PL400 treated SL soil followed by 40.4% increase in SM400 treated in the same soil. The P uptake was significantly enhanced for all manure biochar treatments compared with the Control and WC biochar in SL, while only PL400 and SM400 treatments increased P uptake in the SY soil. The increase in P uptake up to 161% compared to the Control was recorded in PL400 treated SL followed by 119% with the SM400 in the same soil. Similarly, K uptake was significantly enhanced for all manure biochar treatments compared to the Control in the SL soil, while only PL400, PL600 and SM400 treatments increased K uptake in the SY soil. Up to 210% increase in K uptake compared with the Control was observed in PL400 treated SL soil followed by 142% increase in PL400 treated SY soil. The WC biochar had no effect on NPK uptake compared to the Control.

The N UEs, with respect to the total N supplied via biochar, for PL400 and SM400 treatments were greater compared with the other biochar treatments in the SL soil, while higher efficiency was recorded for only SM400 treatment in the SY soil (Table 3). The P UEs for PL400 and SM400 treatments were greater compared with the other treatments in both soils, with the highest value for PL400 treated SL soil followed by SM400 treatment in the same soil. In contrast to these, the highest K UE was recorded in the WC treated SL soil followed by SM400 treatment in the same soil, and the lowest K UE for the SM600 treated SY soil. The NPK UEs, over all treatments, in the SL soil were two times greater than in the SY soil. Significant positive correlations existed between ryegrass N uptake and biochar N contents (n=15, $p<0.01$, r=0.65 for SL and r=0.71 for SY), P uptake and biochar P contents (n=15, $p<0.01$, r=0.68 for SL and $p<0.05$, r=0.50 for SY), as well as K uptake and biochar K contents (n=15, $p<0.01$, r=0.66 for SL and r=0.70 for SY).

3.2. Soil chemical properties

After the last ryegrass harvest, significant effects on all soil properties as a result of biochar additions to both soils were observed apart from soil CEC. The increase of soil C content from a mean of 1.29% in the Control to 2.74 % in the WC treatment was recorded in the SL soil, while the increase remained between 0.91 (Control) and 2.72% (WC) in the SY soil (Table 4). The highest increase of soil C content by 198% compared to the Control was calculated for the WC treated SY soil followed by a 116% increase for the SM400 treated same soil. The mineral N concentrations in both soils after ryegrass harvest were very low (3 mg N kg^{-1}, on average), without differences among treatments. Similarly, a significant increase in the total soil N content

from a mean of 0.13% in the Control to 0.23% in the PL400 treatment was noticed in the SL soil, while such an increase was observed between 0.06% (Control) and 0.15% (PL400) in the SY soil. The WC biochar had no effect on the total N content compared to the Control in either soils. All manure biochars significantly increased the soil pH compared with the Control, while WC had no effect on pH in both soils. An increase in pH from 6.5 pH unit in the Control to 7.88 pH unit in PL600 and SM400 was observed in the SL soil, while pH varied between 8.59 and 9.21 in the SY soil.

Manure biochars caused a significant increase in available P, from a mean value of 20.8 mg kg^{-1} for the Control to a mean of 106.4 mg kg^{-1} for the SM600 treatment in the SL soil (Table 4). The P content in the SY soil was also significantly higher for the manure biochars compared with the Control and WC treatments. Exchangeable K increased significantly only for the PL600 and SM400 treatments in the SL soil, however, while all manure biochars increased exchangeable K in the SY soil. Both exchangeable Ca and Mg significantly increased for all manure biochar treatments in the SL soil. In the SY soil, none of the biochars had an effect on exchangeable Ca, instead a slow steady decline in Ca content was observed relative to the Control, however exchangeable Mg increased significantly with the addition of manure biochars. The CEC failed to vary significantly among the biochar treatments in either soils (Table 4).

3.3. Soil microbial properties and community structure

In the SL soil, the MBC values were significantly lower (p<0.05) compared with the Control for the PL600, SM400 and SM600 treatments, while they were significantly higher for all

biochar treatments including WC in the SY soil (Table 4). The MBC values, on average over all treatments, did not differ between two soil types. The DA significantly increased compared with the Control for the PL400, SM400, SM600 treatments in the SL soil, while this was true only for the PL400 treatment in the SY soil. On the other hand, significantly lower DA for WC treatment compared to the Control and manure biochar treatments was observed in the SL soil, however in the SY soil, this was true for the WC, PL600 and SM600 treatments. The average DA value, over all treatments, was 1.8 times higher in the SL soil than in the SY soil. The DA, in both soils, was significantly positively correlated with the VM contents of the biochars (n=15, $p<0.05$, r=0.61 for SL and $p<0.01$, r=0.91 for SY), but negatively correlated with the biochar C:N ratios (n=15, $p<0.01$, r= -0.82 for SL and r= -0.80 for SY).

The GA was found to be significantly lower for the PL400 and SM400 treatments compared with the Control and WC treatments in the SL soil, and this was also true for the PL400, PL600 and SM400 treatments in the SY soil. Significant negative correlation was observed between GA and the VM contents of the biochar (n=15, $p<0.05$, r= -0.61 for SL and $p<0.01$, r= -0.68 for SY), but a positive correlation between GA and the biochar C:N ratios (n=15, $p<0.01$, r=0.81 for SL and r=0.67 for SY). Over all treatments, GA was on average 1.6 times lower in the SL soil than in the SY soil. The correlation between DA and GA was significantly negative (n=18, $p<0.01$, r= -0.84 for SL and $p<0.05$, r= -0.53 for SY). Moreover, soil pH was positively correlated with DA but only in the SL soil (n=18, $p<0.05$, r=0.57), but was negatively correlated with GA in both soils (n=18, $p<0.05$, r= -0.55 for SL, $p<0.01$, r= -0.65 for SY).

Permutation testing of the bacterial DGGE community profiles revealed a significant separation between the Controls and all biochar treatments in both soils ($p \leq 0.05$). Furthermore, significant differences were found in SL between treatments WC and SM, WC and PL, as well as PL and SM. However, significance was not reached between the two different manure pyrolysis temperatures 400 and 600°C. (Fig. 2a and 2b). In SY, significant differences between the two PL and between PL and WC were found, SM was not significantly different from WC and no significant difference was found between the two SM treatments. Canonical correspondence and permutation analysis of SL identified a significant influence of the soil pH, SOC, TN, C/N ratio and shoot DM yield on the bacterial community structure. Likewise, the same significant influences were identified in SY, with the exception that variations in SOC wasn't significantly influencing the bacterial community structure (Fig. 2a and 2b, arrows indicate significant environmental factors).

4. Discussion

The production of biochars from the manure is of scientific interest as a mean to stabilize the OM in the feedstock, especially where there is an over application of manure and digestates; land application is restricted due to excessive amount of nutrients already present in the soil and their export is limited due to the national legislation. The technical and economic feasibility of producing biochar from pre-treated swine manure and poultry litter has been modelled and simulated based on experimental data (Wnetrzak et al., 2013; Huang et al., 2015). The results of these modelling exercises suggest that the moisture content of the feedstock plays a key role in determining the economic viability. Poultry litter can be supplied to the reactor directly without

any pre-treatment because the relatively low moisture content of such feedstock makes it suitable for pyrolysis (Lynch et al., 2013; Huang et al., 2015). Pig slurry, on the other hand, has to be separated into solid and liquid fractions, as it has a very low dry matter content (Wnetrzak et al., 2013).

At operating temperatures between 300°C and 700°C, most inorganic compounds including essential plant nutrients such as P, K, Ca, Mg etc. can be recovered (100–80%) in the char, even though the loss of some N and S is unavoidable (Cantrell et al., 2012; Van Zwieten et al., 2013). Previous studies reported that the recoveries for C (70–30%), N (75–20%) and S (65–20%) vary within the pyrolysis temperatures (300–700 °C) and among the type of manure feedstocks (e.g. poultry/turkey litter, swine/dairy/horse manure etc.) (Cantrell et al., 2012).

This study showed a remarkable and positive effect of low temperature poultry biochar, both on crop growth and soil chemical properties and subsequent alterations in soil microbial properties. To a minor extent, this was also true for the SM400 but only in the finer textured SL soil. The results of biochar response to crop growth from previous studies are quite variable but a majority of them reported positive yield enhancement (Chan et al., 2007, Van Zwieten et al., 2010; Uzoma et al., 11; Wang et al., 2012b; Zhang et al., 2012; Cornelissen et al., 2013, De La Rosa et al., 2014, Fox et al., 2014, Lin et al., 2015), some studies with no effect at all on yield (Bargmann et al., 2013, Cornelissen et al., 2013, Lin et al., 2015), and a few studies showed negative effects on plant growth, such as Deenik et al., 2010 on macademia nut-shell charcoal, Wisnubroto et al., 2011 on sewage-sludge biochars and Bargmann et al., 2013 on hydrochar. The same biochar used in the same soil in an experiment for two different crops can achieve different

results on crop growth performance as Lin et al. (2015) reported increased wheat yield on maize stalk biochar amended loamy soil (16 Mg ha^{-1}), but found no effect on soybean growth for the identical treatment. This also suggests that crop response to biochar varies with crop types, biochar application rates and properties, growing conditions and edaphic factors (Jeffery et al., 2011).

The higher DM (both shoot and root) yield of ryegrass, in both soils, particularly from low temperature manure biochar treatments may be due to: (i) direct nutrient additions from these chars into the soil-biochar matrix (Lehmann et al., 2003), (ii) higher N mineralization from these biochar amended soils (Ameloot et al., 2015), (iii) improved bioavailability of soil-biochar-P associated with increase in soil pH (in the SL soil) followed by enhanced P uptake plus efficiency (Wang et al., 2012b; Fox et al., 2014), and (iv) improved soil biogeochemical S cycling attributed to the activity of soil biota (Lehmann et al., 2011, Fox et al., 2014; Subedi et al., 2016).

We observed increased N uptake followed by an enhanced N uptake efficiency in the ryegrass plants for the low temperature manure biochar treatments in the SL soil and with all manure biochar treatments (except SM600) in the SY soil. Clough et al. (2013) reported that the low temperature manure- and biosolids-derived biochars contain substantial amount of hydrolysable organic N such as amino acids that could be mineralized microbially, thus would become plant available indirectly or could be even taken up by plant roots directly. This hydrolysable N decreases with increasing pyrolysis temperature (Hossain et al., 2011; Wang et al., 2012a). The positive correlation between N uptake and biochar N content suggested that a substantial

proportion of N from these chars was indeed ultimately plant available (De la Rosa and Knicker, 2011). Additionally, increase in shoot and root DM yield for PL400 and SM400 treatments compared with the Control was accompanied by the enhanced N uptake in the respective treatments in the SL soil. On the other hand, a significant increase in N uptake for PL600 and SM400 treatments, in the SY soil did not necessarily increase shoot and root DM yield compared with the Control. This indicates that soil characteristics also play a role and biochar is more effective in an acidic soil because of its neutralization effect (Deenik et al., 2010; Fox et al., 2014; Lin et al., 2015). Furthermore, the low CEC and OM as well as strong soil alkalinity could be the additional causes for the lack of DM response to the SY soil, leading to restricted plant growth.

We further noted that the causality of biochar derived plant growth promotion was not solely determined by the N uptake but also included enhanced P and K uptake (Van Zwieten et al., 2013), as well as other beneficial cations (Ca, Mg etc.) as significant positive correlations also existed between P, K uptake and biochar P, K contents. The significant increase in P uptake along with the positive correlation corroborates that biochar P is directly or indirectly available to the crops (Wang et al., 2012b; Van Zwieten et al., 2013). Like for N, the higher P UE with PL400 and SM400 treatments in the SL soil also indicates that available fraction of P, associated with enhanced mineralization/mobilization of organic P, is greater in low temperature manure biochars than in the high temperature biochars (Van Zwieten et al., 2013; Jensen, 2013a, 2013b).

The WC biochar in this study did not show an improvement in plant growth, but on the other hand did not reduce the growth with respect to the Control. This indicates that this commercial

biochar could show a positive effect on C sequestration but it needs NPK fertilization when applied to crop (Steiner et al., 2007; Deenik et al., 2010; Van Zwieten et al., 2010; Subedi et al., 2016). Nutrient poor biochars may still act as soil conditioners, altering soil chemical and soil microbial properties resulting in increased soil nutrient uptake that were not available in otherwise acidic soils (Van Zwieten et al., 2010; Lehman et al., 2011; Fox et al., 2014; Gul et al., 2015). On the other hand, the PL400 biochar used in this study had the highest VM and N contents, and the lowest C:N ratio of all biochars. Thus, the N mineralization on such biochar-amended soil is also expected to be higher due to the low C/N ratio and this was fully evidenced by the higher N uptake. The harmful effect of manure biochars due to their high VM contents on ryegrass growth was not visible in our study, in contrast with previous studies which found reduced lettuce and maize growth on macadamia nut shell charcoal-amended soil (Deenik et al., 2010), reduced ryegrass growth on biosolid biochar amendments (Wisnubroto et al., 2011) and inhibited wheat seed germination on hydrochar amended soils (Bargmann et al., 2013). This suggests that not all biochars are harmful to the crops, toxicity being determined only by specific combination of feedstock and processing conditions (Spokas et al., 2011). We also noticed higher concentrations of heavy metals (Zn, Cu, Mn and Ni) in the PL biochars compared with other biochars (Table 2). Nevertheless, the heavy metal concentrations for all biochars used in our study are below the range of maximum threshold values set by different biochar certification schemes (EBC, 2012; IBI, 2014; Domene et al., 2015).

The increase in nutrient (both macro and micro) contents of biochar-amended soils is mainly associated with the direct addition of these elements (DeLuca et al., 2006; Subedi et al., 2016). An expected significant increase in soil C contents with biochar additions in both soils showed

the potential of such biochars in sequestrating C in the long run. Such a large increase in soil C contents can also be explained due to the higher root biomass turnover associated with increased biomass production as result of soil biochar addition (Kammann et al., 2012). A significant increase in the total N contents with the PL400 and PL600 treatments showed that this N could successively be available, via microbial mineralization, for the subsequent crops (Clough et al., 2013). However, the mineral N fraction of all treatments were too low at the end of the ryegrass harvest, indicating that the plants were able to up take almost all of the available N forms.

All the biochars significantly increased the soil pH compared with the Control in both soils. The increase in pH of the slightly acidic SL soil is a positive liming effect towards soil neutralization (Fox et al., 2014); however this pH increase in the already alkaline SY did not play any negative role in terms of ryegrass DM yield. None of the biochars in this study improved soil CEC. Even though the contact between soil and biochars was at least 1 year (Subedi et al., 2016), they were not successful in enhancing the CEC. Organic matter in biochar feedstock often loses its functional groups during pyrolysis and they can rebuild again over time as result of biochar oxidation into soils, but this may take several years to see the actual effect on soil CEC (DeLuca et al., 2006, Singh et al., 2010).

The available P contents in soil-manure biochar matrix in both soils, after ryegrass harvest, were significantly higher compared with the Control providing further evidence that a large fraction of biochar P was available to the crop and these materials can also act as a P fertilizer (Wang et al., 2015a). We can also notice that the significant amount of residual P after ryegrass harvest may also lead to P overload in the soil eventually contributing to environmental problems

(e.g. eutrophication, leaching). The exchangeable K contents was significantly higher than the Control only for the PL600 and SM400 treatments in the SL soil, while it was higher for all manure biochar treatments in the SY soil. The average K content, over all treatments, in SL soil was 1.6 times lower than in the SY soil and was linked to the high K uptake in the SL soil. We noticed that the plant was able to deplete more K from the SL soil than from the SY soil as SL soil had high DM yield. Furthermore, Ca may have competed more with K for the exchange sites in the SL soil (except PL600 treatment) due its higher affinity as the Ca availability is relatively higher in SL soil than in SY soil, while opposite can be true for SY soil (Sachs, 2004). It is also true that a near neutral pH normally reduces the amount of K in the soil solution, as the SL soil used in this study had pH range of 6.5–7.8 (Sachs, 2004).

The exchangeable Ca content was significantly higher than the Control only for the PL400 treatment in the SL soil, while no biochars had an effect on Ca content in the SY soil despite the high input with all biochars. The solubility of Ca and consequently its availability is mainly determined by the soil pH and soil P content. The low exchangeable Ca content of manure biochar treated SY soil is explained by the binding of Ca with biochar P resulting into the formation of insoluble calcium phosphates as the total P supplied through manure biochar into the soil was significant (up to 575 mg P kg^{-1} with PL600 treatment) (Cui et al., 2011; Wang et al., 2015a). Since the SY soil used in this experiment was highly calcareous (15% $CaCO_3$), significant amount of Ca is unexchangeable as this could have been precipitated as insoluble $CaCO_3$ (Wojtowicz, 1998). Furthermore, we observed that the low exchangeable Ca in SY soil is also regulated by the soil pH as the Ca availability starts decreasing slowly pH above 8.5 and biochar treated SY soil in this study ranged between 8.5–9.2 (Wojtowicz, 1998; Sachs, 2004).

Insoluble phosphate salts could subsequently be made available for the crops through the phosphate mobilizing bacteria or root exudation of organic acids (Gyaneshwar, 2002).

The result of the MBC in this experiment seems to be completely different in the two soils. In the SY soil, all biochars significantly increased MBC compared with the Control. The mechanisms that might have resulted in the increased MBC from the biochar amendments in this soil include metabolism of labile organic C compounds in the biochars (Bruun et al., 2008; Smith et al., 2010; Bruun et al., 2011), microbial access to nutrients on biochar surfaces (Cheng et al., 2008), enhanced microbial activity due to rapid decomposition of SOM (Wardle et al., 2008), and physical protection for microbes as biochar pores and surfaces could serve as habitat for the microbes (Lehmann et al., 2011). In contrast, the MBC values for PL400 and WC treatments did not differ significantly compared to the Control in the SL soil. A possible explanation could be that the addition of labile C through the biochars might have led to no noticeable significant effect on the MBC as the SL soil was characterized as rich in OM. Surprisingly, the MBC values were significantly lower for the PL600, SM400 and SM600 compared with the Control in the SL soil. It is possible that a mechanism other than, or in addition to, soil organic C content may have been responsible for the significantly lower values of MBC exhibited by these biochar amended soil. This is beyond the scope of this research. Since significant plant growth promotion was recorded under the SM400 (compared with the Control) and PL600 (compared with the WC) treatments, the role of soil microbial biomass could have been less important than direct nutrient supply in respective biochar treatments. Therefore, our results of MBC beg further experimental analytical verification.

The dehydrogenase is an intracellular enzyme and is likely not to be affected much by the quorum sensing, the so called production and release of the chemical signal molecules (Masiello et al., 2013). The readily available C components (e.g. simple soluble sugars, volatile organic compounds) of the low temperature manure biochars (PL400 and SM400) could stimulate the heterotropic microbial activity and subsequently increase the DA (Van Zwieten et al., 2013, Ameloot et al., 2014, 2015). As the DA was positively correlated with VM content and negatively correlated with C:N ratios of the biochars in both soils, this supports the idea of enhanced microbial consumption of volatile C triggering such enzyme activity as suggested by Ameloot et al. (2015). The lower DA with WC biochar treatment (in both soils) and with PL600, SM600 treatments (in the SY soil) compared to the Control could be explained by substrate blocking or adsorption into high temperature biochar surfaces (Ameloot et al., 2014; Bailey et al., 2011). In addition, it also indicates that the high temperature biochars are more recalcitrant as well as resistant against microbial attack, showing the potential of these biochars in sequestering C in the long-run (Subedi et al., 2016). The correlation between soil pH and DA was also significantly positive in the SL soil, while this was not in the SY soil though positive, suggesting that the liming potential of manure biochars is of importance in stimulating DA in the SL soil (Ameloot et al., 2015).

The β-glucosidase is an extracellular enzyme and is likely to be affected by the quorum sensing chemicals (Masiello et al., 2013). Increased activity of this enzyme fosters further microbial activity when initial pools of labile C compounds (e.g. glucose) are consumed rapidly by the microbes, thus targeting on high molecular weight C compounds (e.g. lignin and cellulose) mainly due to the change in substrate use patterns (co-occurrence) of soil microorganisms as a result of

biochar additions (Eivasi and Tabatabai, 1988, Lehmann et al., 2011). The stimulation of such enzyme is considerably affected by the accumulation of labile organic compounds resulting in its catabolic repression due to high level presence of glucose (Ameloot et al., 2014; Kotroczó et al., 2014). The negative correlation between GA and the biochar VM contents would support this hypothesis on stimulation of such enzymes.

The higher level of GA for the Control treatments in both soils can be explained by the presence of high molecular weight organic compounds (e.g. polymers) in the native SOM, showing a dominant role of GA over DA in breaking down the cellulosic C into simple sugars such as glucose. The correlation between GA and soil pH was also negative in both soils. Moreover, an increase in DA in both soils was accompanied by the decrease in GA and vice versa. This was supported by the negative correlation between DA and GA in both soils, suggesting that these two enzymes act differently based on substrate availability, their sorption and desorption into biochar surfaces and their differing response is considerably affected by the presence of labile organic matter in the soil biochar mixture (Spokas et al., 2011; Kotroczó et al., 2014; Ameloot et al., 2015). Additionally, a significant positive correlation between GA and biochar C:N ratios further suggests that lacking labile C substrate reduces DA activity, but increases GA as an alternative means of providing C as food for the microbes (Awad et al., 2012; Ameloot et al., 2014; Wang et al., 2015b). In general, these two enzymes provide insights on the degree of resistance of organic matter against microbial degradation in soils amended with biochars. They show that labile C availability is higher in manure-derived biochars *vs* standard wood-derived biochar, in poultry manure biochars *vs* swine manure biochars, in low *vs* high pyrolysis temperature (Lehmann et al., 2011; Award et al., 2012).

While only some biochar applications had a significant effect onto plant growth, soil nutrient status, soil enzymatic activities and nutrient uptake, all soil bacterial communities were significantly affected by the biochar deposition as revealed by the CCA and permutation analysis, including WC. In contrast, pyrolysis temperature had a limited impact on the bacterial communities as only PL400 and PL600 could be significantly separated in SY. Significant changes in bacterial community structures may result into beneficial effects to soil nutrient availabilities, when key nutrient mobilizing bacteria increase in abundance or activity as for instance identified by Fox et al. (2014). There, higher abundances of both S- and P-solubilizing bacteria were found in biochar treated soil of low P availability alongside significant contributions of such bacteria in shifting the community structure. Likewise, aromatic sulfonates present on the manure biochars could also have been desulfurized by desulfonating bacteria as Schmalenberger and Noll (2014) found a significant response of these bacteria to aromatic sulfonate addition in grassland soils. Future investigations in phosphatase and sulfatase activities on enzymatic and gene expression levels may close this remaining knowledge gap (Schmalenberger and Fox, 2016). In this study, shifts in bacterial community structures were significantly correlated with changes in soil pH, soil C, TN, C/N ratio and plant growth. Physico-chemical changes in the amended soils including pH may explain some but not all of the observed community shifts (Jones, 2009; Lehmann et al., 2011) as no pH change in WC amendment was detected in both soils. In SL, effects of significantly high shoot DM (in PL400 and SM400) were not reflected in significantly altered bacterial community structures. However, differences were found in SY, where significant increases in shoot and root DM (in PL400) were correlated to a shift in the bacterial community structure. These findings suggest that the altered

bacterial communities of PL400 amendments in SY may have contributed to increased nutrient mobilization and plant growth after all.

Although this study excludes non-pyrolyzed manure (raw feedstock) as a reference treatment for comparison, previous study reported that pyrolysis of manure (poultry litter) resulted a biochar with higher C stability associated with greater aromaticity as well as higher P and K fertilizer value than the raw feedstock, potentially adding both agronomic and environmental benefits (Van Zwieten et al., 2013). In addition to these benefits, we recommend further investigation of the economic potential of this pyrolysis technique compared with other technologies, such as composting and anaerobic digestion of manure feedstock in agricultural sector considering a life cycle analysis of the entire system.

5. Conclusions

The total DM yields of ryegrass (both above and belowground biomass) were related to both positive nutrients content (NPK and cations) of biochars and enhanced soil characteristics (chemical and biological). This shows that low temperature manure biochars (PL400 and SM400) can be utilized as potential NPK-fertilizers with a significant value as most of the nutrients can be recovered into the char. None of the biochars were found to be toxic for the ryegrass growth as they all boosted the growth, despite being high in VM content. All biochars significantly increased the soil C contents compared to the Control after ryegrass harvest, with WC biochar showing the greatest C sequestration potential. All manure biochars showed a positive liming effect in acidic silt-loam soil, and increased nutrient availability (except Ca) in both soils.

Despite manure biochars showed positive enhancement in relation to yield and soil fertility (both chemical and biological), they were not successful in compensating differences between soil type. The more fertile soil enhanced the possibility to show differences among biochar types. As this work mainly focussed on assessing the fertilizing value of manure biochars at a small scale lab experiment, field trials with careful considerations of environmental issues (e.g. eutrophication, leaching) due to potential excessive nutrient load (mainly P and Ca) are required before applying such materials in the field for making specific agronomic recommendation. In addition, further investigations looking into the technical and economic viability of manure pyrolysis in relation to nutrient flow and recovery, plus a feasibility study compared to other manure management practices, such as composting or anaerobic digestion, are also recommended.

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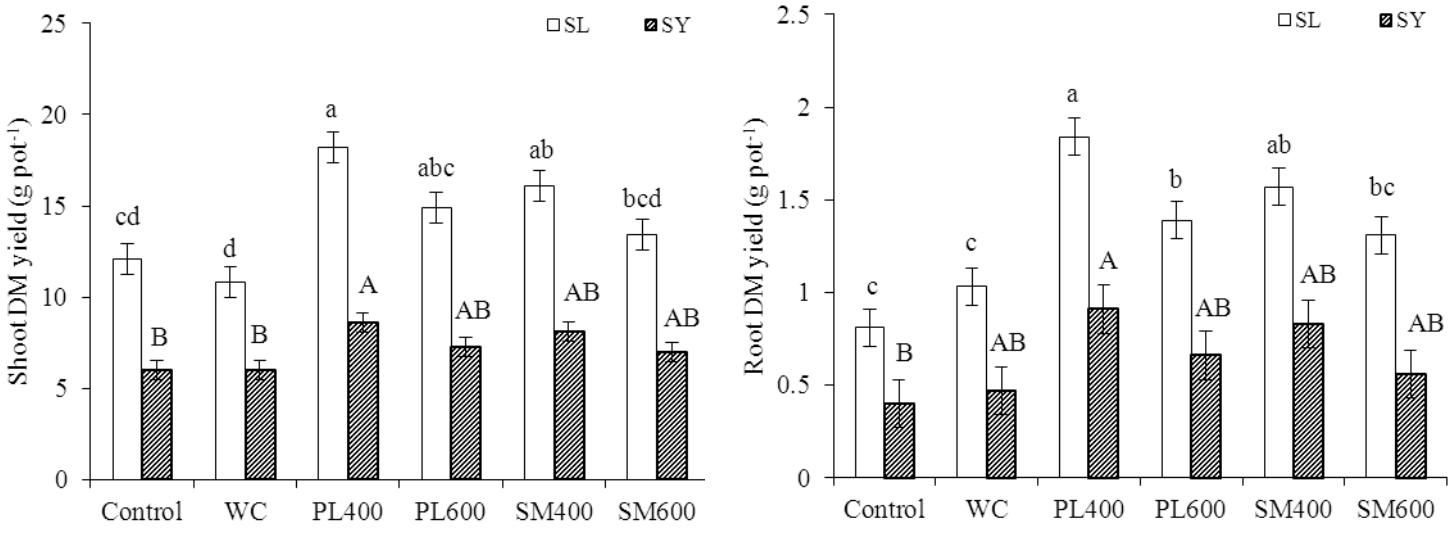

Fig. 1. Effects of biochar amendment on above (graph on the left) and belowground (graph on the right) biomass yield. Error bars represent standard error of the means (n=3). Please note different scales of Y-axis. Different small letters indicate significant differences (p<0.05) in SL soil (empty bars), while capital letters indicate differences (p<0.05) in SY soil (striped bars) between different treatments for shoot and root DM yield.

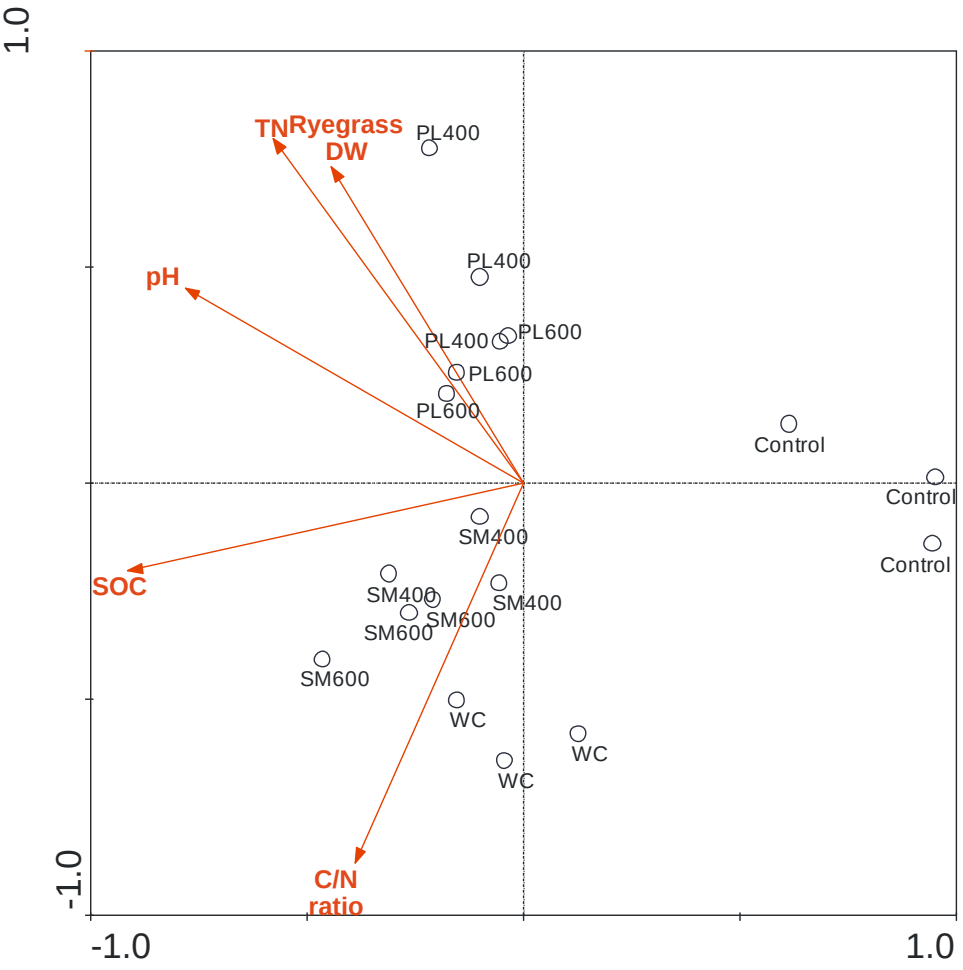

Fig. 2a. CCA plot showing the effects of biochar amendments on 16S rRNA gene based bacterial community structure in soil-loam (SL) soil (n = 3) with soil pH, SOC, TN, C/N ratio and ryegrass yield (DW) defined as environmental factors. Arrows for each variable tested indicate significance ($p \leq 0.05$, permutation test) of environmental factors on shift of the bacterial community structure upon biochar amendment.

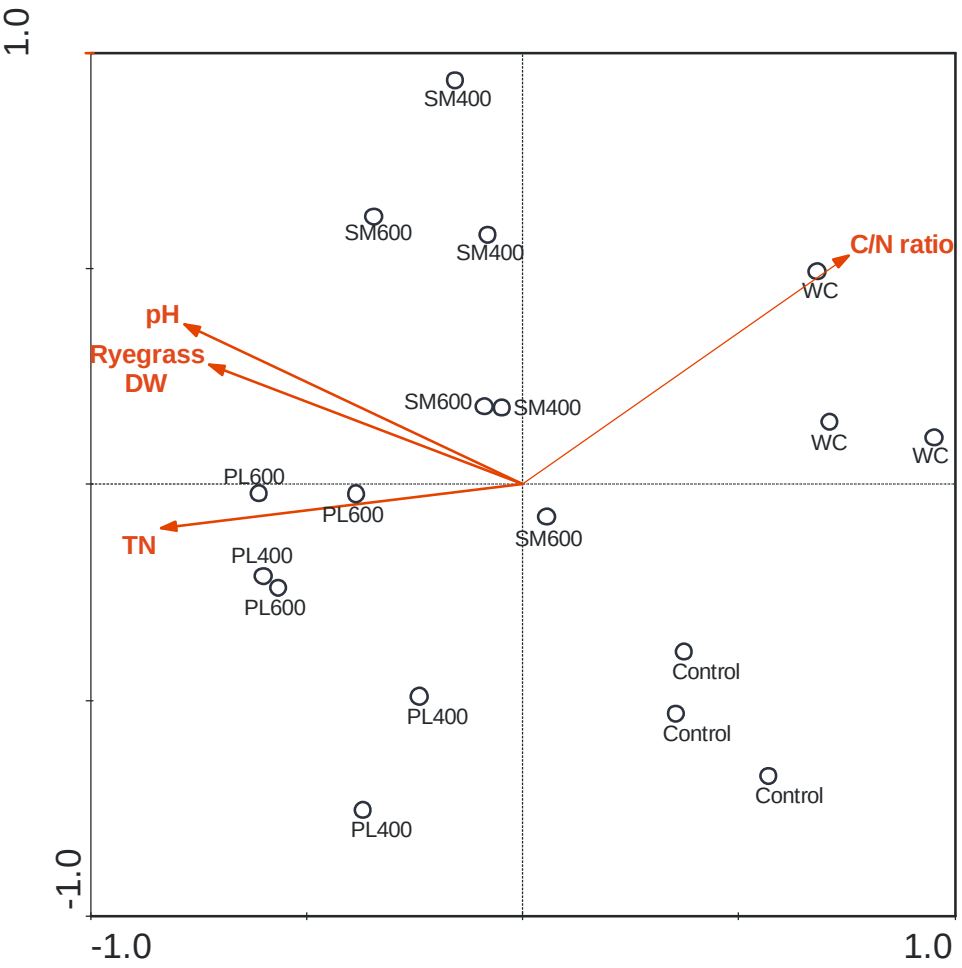

Fig. 2b. CCA plot showing the effects of biochar amendments on 16S rRNA gene based bacterial community structure in sandy (SY) soil (n = 3) with soil pH, TN, C/N ratio and ryegrass yield (DW) defined as environmental factors. Arrows for each variable tested indicate significance ($p \leq 0.05$, permutation test) of environmental factors on shift of the bacterial community structure upon biochar amendment.

Table 1

Properties of the soils used in the experiment.

Soil type	Sand	Silt	Clay	Porosity[a]	$CaCO_3$	SOC	TN	C/N	pH	CEC	P[b]	K[c]	Ca[c]	Mg[c]
	(%)							–	–	($cmol_c\ kg^{-1}$)	($mg\ kg^{-1}$)			
SL	17.2	71.1	11.7	49.2	0.4	1.2	0.15	8.0	6.1	12.4	23	42	1452	179
SY	89.7	5.5	4.8	45.3	15.3	0.52	0.057	9.1	8.3	5.4	14	28	980	21

Abbreviations: SL = silt-loam, SY = sandy, SOC = soil organic carbon, TN = total nitrogen, CEC = cation exchange capacity.

[a] calculated on the basis of bulk density and particle density.

[b] available (Olsen P).

[c] exchangeable.

Table 2

Physico-chemical characteristics of different biochars utilized in the experiment.

Biochar type[a]	TC (%)	TN	VM	Ash	C:N –	CEC ($cmol_c\ kg^{-1}$)	P[c] ($g\ kg^{-1}$)	P[b]	Ca[c]	Mg[c]	K[c]	pH –	Fe ($mg\ kg^{-1}$)	Al	Zn	Cu	Mn	Ni	Pb	SA ($m^2\ g^{-1}$)	PV ($cm^3\ g^{-1}$)
PL400	52.1	5.85	44.9	25.3	9.0	30.2	20.0	12.3	28.3	17.3	38.8	9.5	2909	537	1164	349	1099	52	13	5.4	0.006
PL600	52.8	4.01	24.7	35.4	13.0	27.5	28.7	15.4	35.9	24.0	58.8	10.4	4311	777	1633	366	1437	52	13	6.3	0.012
SM400	54.9	2.23	29.9	27.5	24.6	52.5	22.1	9.7	20.3	15.7	16.2	10.0	5392	617	585	156	455	26	bdl	5.8	0.009
SM600	57.9	1.79	17.8	34.5	32.4	18.6	28.2	15.6	28.9	21.3	35.3	10.4	6674	834	770	180	513	26	13	10.6	0.01
WC	89.3	0.27	15.3	7.8	335.4	14.8	0.92	0.7	13.6	3.2	2.6	11.0	1322	1097	79	53	397	40	13	178.3	0.14

Abbreviations: Same as given in Table 1 as well as: TC = total carbon, VM = volatile matter, SA = surface area, PV = pore volume, bdl = below detection limit.

[a] Letters refer to feedstock material as poultry litter (PL), swine manure (SM) and wood chip (WC), numbers refer to pyrolysis temperature in ºC, with addition to WC at 1000 ºC.

[b] Available.

[c] Total.

Table 3

Effects of biochar amendments on above ground nutrient uptake and uptake efficiency on two soils (n = 3).

	Above ground uptake (mg pot^{-1})			Uptake efficiency[a] (%)		
Treatment	N	P	K	N	P	K
			SL soil			
Control	478.1 c	47.3 c	304.1 d			
WC	457.9 c	37.6 c	369.1 cd	-24.8[b]	-44.7	83.4
PL400	785.9 a	123.7 a	943.2 a	17.5	20.8	54.9
PL600	511.2 bc	96.5 ab	807.1 a	2.7	10.6	28.5
SM400	671.6 ab	103.6 ab	629.1 b	28.9	19.3	66.9
SM600	460.2 c	87.3 b	451.2 c	-3.3	8.6	13.9
SE	46.6	7.33	37.1			
p	0.001	<0.001	<0.001			
			SY soil			
Control	209.9 b	25.1 b	218.1 d			
WC	204.8 b	22.5 b	262.5 cd	-6.3	-11.6	57.0
PL400	291.8 a	49.8 a	527.3 a	4.7	6.7	26.6
PL600	287.5 a	36.5 ab	484.4 a	6.5	2.5	15.1
SM400	285.1 a	44.4 a	337.5 bc	11.2	6.7	24.6
SM600	227.1 ab	33.8 ab	303.4 cd	3.2	1.9	8.1
SE	15.4	4.32	29.8			
p	0.003	0.005	<0.001			

Abbreviations: Same as given in Tables 1 and 2 as well as: SE = standard error of the mean.

Mean values followed by the same letter are not significantly different ($p<0.05$).

[a] (Treatment – Control)/NPK supplied via biochar * 100, respectively for N, P and K uptake.

[b] Calculated based on average uptake values for each treatment per soil type.

Table 4

Effects of biochar amendments on chemical and microbial properties of silt-loam (SL) and sandy (SY) soils (n = 3) at the end of the ryegrass growth experiment.

Treatment	C[a]	TN	pH	P[b]	Ca[c]	Mg[c]	K[c]	CEC	MBC	DA[d]	GA[e]
	(%)		–	(mg kg^{-1})				($cmol_c$ kg^{-1})	(µg g^{-1})	(µg g^{-1} h^{-1})	
						SL soil					
Control	1.29 b	0.13 c	6.56 c	20.8 e	1890.1 b	246.9 d	10.8 c	16.1	186.6 a	48.6 c	187.7 ab
WC	2.74 a	0.14 c	6.81 c	20.8 e	2010.2 b	253.5 d	24.7 c	16.3	167.2 ab	18.4 d	215.1 a
PL400	2.12 a	0.23 a	7.84 a	98.2 b	2347.1 a	281.2 c	37.8 bc	16.5	189.2 a	89.1 a	127.1 cd
PL600	2.29 a	0.21 ab	7.88 a	89.2 c	1890.4 b	311.3 b	119.4 a	14.6	109.1 bc	56.8 bc	149.5 bc
SM400	2.45 a	0.18 abc	7.36 b	77.1 d	1899.4 b	331.9 b	57.5 b	16.4	45.1 c	92.2 a	92.2 d
SM600	2.43 a	0.17 bc	7.88 a	106.4 a	1999.8 b	372.1 a	22.2 c	16.0	58.5 c	80.1 ab	152.1 bc
SE	0.14	0.01	0.10	1.35	68.3	5.46	6.14	0.90	15.6	6.51	9.21
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.00	0.710	<0.001	<0.001	0.001
						SY soil					
Control	0.91 c	0.06 c	8.59 c	12.6 d	1042.1	84.6 c	18.1 d	6.93	67.6 c	48.4 b	288.2 a
WC	2.72 a	0.08 bc	8.64 c	14.2 d	1027.5	87.9 c	9.4 d	6.49	150.7 ab	4.88 d	310.7 a
PL400	1.66 b	0.15 a	8.83 b	108.1 b	862.3	149.4 b	115.1 b	6.89	185.3 a	59.3 a	190.8 b
PL600	1.89 b	0.14 a	9.21 a	136.5 a	885.8	182.7 a	143.8 a	6.76	124.6 b	32.1 c	177.6 b
SM400	1.97 b	0.09 bc	8.99 b	129.3 a	957.9	154.1 b	82.7 c	7.38	114.8 b	46.7 b	187.3 b
SM600	1.75 b	0.11 ab	8.97 b	83.7 c	1018.9	136.2 b	88.1 c	7.78	175.2 a	27.2 c	292.7 a
SE	0.14	0.01	0.03	2.21	46.7	3.97	5.88	0.52	4.43	2.1	16.8
p	<0.001	0.001	<0.001	<0.001	0.072	<0.001	<0.001	0.533	<0.001	0.001	<0.001

Abbreviations: Same as given in Tables 1 and 3 as well as: SE = standard error of the mean, MBC = microbial biomass carbon, DA = dehydrogenase activity, GA = β-glucosidase activity.

Means followed by the same letter are not significantly different ($p<0.05$).

[a] After subtracting inorganic C from the total soil C.

[b] Available (Olsen).

[c] exchangeable.

[d] Expressed as produced level of iodonitrotetrazolium formazan (INTF), and

[e] As produced level of p-nitrophenol (PNP).