RESEARCH ARTICLE

REVISED Crop production under nitrogen starvation conditions: relationships with applied organic matter and soil microbial biomass [version 2; peer review: 2 not approved]

Masato Oda ¹, Uchada Sukchan²

¹Japan International Research Center for Agricultural Sciences, Tsukuba, Japan

²Agricultural Production Science Research and Development Center, Khon Kaen, Thailand

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Abstract

Background: The application of organic matter with a high C/N ratio is effective for the prevention of soil degradation, although this can cause nitrogen starvation. However, some fields are highly productive under nitrogen-starvation conditions. The underlying mechanisms for this are unclear but the correlation between soil microbial biomass (SMB) and crop yield suggests that nitrogen flows from SMB to crops. We aimed to clarify this flow and the source of nitrogen.

Methods: We achieved nitrogen starvation conditions by applying waste mushroom bed and repeated lettuce cropping with different crop management practices, such as watering and fertilizer application. We analyzed correlations among crop yield, SMB, and total soil nitrogen.

Results: The order of the lettuce yield stably corresponded with the management practice used. The SMB increased remarkably by the time of the second lettuce cropping and showed a strong correlation with crop yield. The nitrogen from the waste mushroom bed was lost by denitrification within the crop season. The rate of decomposition showed no correlation with yield or SMB.

Discussions: The crop yield corresponded with the management practice earlier than SMB. Namely, no nitrogen flow from SMB to crop. Furthermore, most applied nitrogen was denitrified and the rate of decomposition (amount of released nitrogen) not affected yield or SMB, so the nitrogen flows of applied organic matter, SMB, and crops are independent. Therefore, the nitrogen source of both SMB and crops is biological fixation.

Conclusions: The correlation between SMB and crop yield is not a causal relationship. The nitrogen source for both is biological nitrogen fixation. The application of organic matter enhances this by occurring nitrogen starvation but not providing a nitrogen source.

Open Peer Review

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1. Jianwei Li , Tennessee State University, Nashville, USA		
2. Oskar Franklin , International Institute for Applied Systems Analysis, Laxenburg, Austria		
Any reports and responses or comments on the article can be found at the end of the article.		

Keywords

Organic farming, Sandy loam, Tropics, Northeast Thailand, Taguchi method

This article is included in the **Agriculture, Food and Nutrition** gateway.

Corresponding author: Masato Oda (oda.masato@affrc.go.jp)

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REVISED Amendments from Version 1

- The descriptions for the decomposition rate of organic matter are improved.
- The methods added more details.
- The date of soil sampling for the middle of season 4 was corrected from day 25 to day 15.
- Table 3 added TC and the change included the input.
- Citations for the K factor and recent work for BNF were added.

Any further responses from the reviewers can be found at the end of the article

Introduction

The application of synthetic nitrogen is a key technology used in agriculture; however, it decreases soil microbial biomass (SMB) and results in soil degradation (Mulvaney *et al.*, 2009). Conversely, the application of organic matter with a high C/N ratio increases SMB and helps degraded soil to recover (Chaudhary *et al.*, 2009; Goyal *et al.*, 1999). The problem that remains is nitrogen starvation (van Iterson, 1904). However, some fields are highly productive under conditions of nitrogen starvation (Nakatsuka *et al.*, 2016; Oda *et al.*, 2014).

The possible mechanism underlying this high productivity under nitrogen starvation conditions is the large flow of low concentrations of nitrogen. Possible sources of this nitrogen include decomposed organic matter, turnover of SMB, and micro-organisms in the root zone (Geisseler *et al.*, 2010). Previous studies have mentioned a correlation between SMB and crop yield (Entry *et al.*, 1996; He *et al.*, 1997); however, these studies also found a correlation between soil total nitrogen (TN) and crop yield at the same extent. Although there is also a study that did not find a similar correlation (Holt & Mayer, 1998). Logically, the correlation between SMB and crop yield occurs in one of three ways: 1) both SMB and crop yield are related to other factors such as TN; 2) SMB is related to crop yield; or 3) crop yield is related to SMB.

In this study, we created nitrogen starvation conditions by applying organic matter with a high C/N ratio (waste mushroom bed) to fields and performed repeated lettuce cropping with different crop management practices. We analyzed correlations among crop yield, SMB, and TN to verify the above three possibilities. In addition, we analyzed the correlation between the rate of decomposition of the organic matter and SMB to verify whether the nitrogen source of the SMB is the organic matter.

We found that the correlation between SMB and crop yield is not a causal relationship and the nitrogen source for both is biological nitrogen fixation. The application of organic matter enhances this by occurring nitrogen starvation but not providing nitrogen source.

Methods

Site description

This study was conducted at a site with a tropical savanna climate in a lateritic loamy sand field in Tha Phara village, Khon Kaen Province, Thailand (16° 34' N, 102° 83' E), during 2011

and 2012. The site had ideal conditions for this study because sandy soils at a high temperature have a low level of soil organic matter and high microbial activity. Conducting the study during the dry season avoided any effects from rainwater.

Treatments

We chose waste mushroom bed (gifted by a local farmer) as an organic material because of its adequate C/N ratio (40) for producing nitrogen starvation and its homogeneity. We applied the material to the fields at a fresh-weight rate of 1 kg m⁻² (equivalent to a C application rate of 300 g m⁻², equivalent to 30% of the initial topsoil) and mixed it well with the topsoil (about 14 cm) at each time of planting. This ensured that the nutritional input was the same for all plots. We generated a gradient of SMB and crop yield by altering crop management practices, including watering (1.5 mm twice per day, 3 mm once per week, or none); urea application with mushroom waste as a starter of decomposition (at a rate of 10, 0.1, or 0 g m⁻², the 0.1 g m⁻² application performed as a solution in 1.0 l of water); waste mushroom bed application method (incorporated, applied to the soil surface, or incorporated after killing the fungi by packing the material in plastic mulch film and exposing it to sunlight for 1 d); and planting density (standard, double, or none). No other materials were used.

The four factors of the three levels of practice (Table 1) were assigned to an L₁₈ orthogonal array (Taguchi, 1986). Such a design enables each effect to be evaluated at an accuracy of six replications. The plot (3 × 3 m) locations were first randomized then fixed.

The practice of plant density 0 was used to verify the independence of SMB from crop growth in the experiment, because an increase in the size of the root zone is associated with an increase in microbial activity (Alam *et al.*, 2014). The yield of plant density 0 reflects no effect of any practice on the yield, but the problem is evenly allocated to all factors. It should be noted that the aim of the present study was not to evaluate the effects of each factor.

Cultivation

Sequential cropping is thought to be an essential condition for achieving high SMB (Oda *et al.*, 2014). Therefore, we planted water spinach during the rainy season (seeded Aug 25, harvested Sep 26), then planted lettuce during the dry season (transplanted and harvested on Oct 20 and Dec 7; Dec 8 and Jan 17; and Jan 23 and Mar 1, respectively). Total precipitation for the first to fourth crop seasons was 248, 7, 0, and 0 mm, respectively. The plants were free from disease and insect pests; no plant protection procedures were used. The field was kept free of weeds by hand weeding.

Determination

We harvested the whole crop of lettuce and immediately oven-dried and weighed it to obtain the dry weight. Topsoil (to a depth of about 14 cm, bulk density 1.22) was sampled from each plot just after the crops were harvested. A composite sample from ten sampling points was collected from the field just before starting treatments. A composite sample from ten sampling points was collected from each plot after harvest. Additionally, sampling was conducted on day 15 of season 4 for checking the decomposition rate in the middle of the crop season. Each soil sample

Table 1. Experimental design using an L_{18} orthogonal array.

Plot No.	Management practice			
	Watering	Type and position of applied material	Plant density	N application (g m^{-2})
	1: 0 2: Once per week 3: Twice per day	1: Alive mixed 2: Alive on soil surface 3: Dead mixed	1: Double 2: Standard 3: 0	1: 0 2: 0.1 3: 10
1	1	1	1	1
2	2	2	2	2
3	3	3	3	3
4	1	1	2	2
5	2	2	3	3
6	3	3	1	1
7	1	2	1	3
8	2	3	2	1
9	3	1	3	2
10	1	3	3	2
11	2	1	1	3
12	3	2	2	1
13	1	2	3	1
14	2	3	1	2
15	3	1	2	3
16	1	3	2	3
17	2	1	3	1
18	3	2	1	2

was sieved through a 2-mm sieve (the mushroom waste could pass through it) while moist, and 500 g of each sample was stored at 2°C until the SMB-N content was measured. The SMB-N content was measured using the fumigation–extraction method (Amato & Ladd, 1988). The inorganic nitrogen concentration of each sample was determined by extracting the sample with 2 M KCl and performing NH_4^+ and NO_3^- assays on the extract (Keeney & Nelson, 1982). The remaining portion of each sample was air-dried, and the total nitrogen and total carbon (TC) content of the soil was determined using an NC analyzer (SUMIGRAPH NC 200F; Sumitomo Chemical, Tokyo, Japan) using the dry combustion method.

Analysis

We analyzed simple correlations (Pearson product-moment) among the crop yield, SMB, and TN using the mean values of the practices (excluding the values of plant density = 0). To do this, we used the CORREL function in Microsoft Excel 2016.

Results

We achieved nitrogen-starved soil by applying waste mushroom bed followed by repeated lettuce cropping under different crop management practices, then analyzed correlations among crop yield, SMB, and TN.

A high yield was achieved under nitrogen starvation

The $\text{NO}_3\text{-N}$ content of the soil during the lettuce crop season was very low ($2.3\text{--}3.1 \mu\text{g g}^{-1}$) compared with the threshold of fertilizer application used for conventional cultivation ($20 \mu\text{g g}^{-1}$) (Breschini & Hartz, 2002; Fox *et al.*, 1989). Table 2 shows the changes in soil properties. The maximum lettuce yield (45 g DM m^{-2}) was higher than the average obtained through conventional farming in Thailand (33 g DM m^{-2} , Department of Agricultural Extension 1996–2001; calculated as 4.1% DM, Food composition table ver. 7). No correlation between soil $\text{NO}_3\text{-N}$ content and yield was found. The order of the yield corresponded with the practices and resulted in correlations among the dry seasons in terms of yield (Figure 1).

Table 2. Yield and soil properties at harvesting.

		Yield ^a as dry matter g m ⁻²				SMB-N ^b content in soil µg g ⁻¹				Total N content in soil µg g ⁻¹				
Crop season		1 ^d	2	3	4	1 ^d	2	3	4	init. ^c	1 ^d	2	3	4
Watering	0	54	10	13	4	78	35	235	231	407	405	382	362	340
	Once per week	59	26	39	22	83	36	399	307		430	397	422	415
	Twice per day	54	38	37	29	76	48	424	283		418	422	362	412
Waste mushroom bed	Alive, mixed	56	29	45	21	85	45	410	319		387	410	383	417
	Surface	44	21	19	17	73	35	323	222		423	380	372	355
	Dead, mixed	67	23	25	16	79	40	324	281		443	410	390	395
Plant density	Standard	70	29	33	21	85	37	369	293		415	437	402	387
	Double	41	20	26	15	69	35	283	278		403	352	367	355
	0					83	47	406	251		435	412	377	425
N application g m ⁻²	0	57	28	20	18	75	32	334	250		347	345	317	328
	0.1	45	18	33	18	91	50	348	264		500	418	380	397
	10	66	27	37	19	72	38	375	308		407	437	448	442
Average		56	24	30	18	79	40	352	274	407	418	400	382	389
Min		41	10	13	4	69	32	235	222	407	347	345	317	328
Max		70	38	45	29	91	50	424	319	407	500	437	448	442

n=6

^a n=4, ^b Soil microbial biomass nitrogen, ^c initial value, ^d rainy season (water spinach).

Fresh waste mushroom bed was applied to every crop. Lettuce was grown in the dry season.

Figure 1. Management and seasonal yields. ○Season 1, ●Season 2, △Season 3, ▲Season 4 Mean values (n=4) for different crop management practices are shown. Season 1 was during the rainy season (water spinach) and the others were during the dry season (lettuce). W: Watering (0: none, 1/w: once per week, 2/d: twice per day); M: Material position (S: surface, A: incorporated, D: incorporated following disinfection by the sun); P: Plant density (1: standard, 2: double); N: Nitrogen application (0: none, 0.1: 0.1g m⁻², 10: 10g m⁻²).

Correlation between SMB and crop yield

The SMB increased remarkably during seasons 3 and 4 (Figure 2). The maximum SMB-N content ($424 \mu\text{g g}^{-1}$) was an order of magnitude larger than that found in previous studies (Entry *et al.*, 1996; He *et al.*, 1997; Holt & Mayer, 1998). The SMB was lower in season 4 than season 3. The low soil moisture content during the later dry season affected the SMB because of the large effect of watering practice (Table 2). The SMB changed largely according to the practice used to input the same quantity of material. SMB showed a strong correlation with crop yield. The correlation ($r = 0.977$, $p < 0.01$) with SMB was stronger than that with TN ($r = 0.588$, $p = 0.06$). The SMB was independent of the crop yield because the SMB-N of plant density = 0 was approximately the average for that of the practices (Figure 3).

Fate of waste mushroom bed

Despite the $1 \text{ kg m}^{-2} \text{ crop}^{-1}$ of waste mushroom bed application (equivalent to 30%, totally 120 %, of the initial topsoil TC contents), TC contents of the initial and the seasonal average were stable (5837, 6071, 5438, 5582, and $5174 \mu\text{g C g soil}^{-1}$). The waste mushroom bed applied decomposed within the crop season. This rapid decomposition of organic material in the sandy soil of the tropics is consistent with the findings of a previous study (Chivenge *et al.*, 2011). Soil TN contents were the same (Table 2). The amount of nitrogen applied was $7.5 \text{ g m}^{-2} \text{ crop}^{-1}$ (equivalent to 10% of the initial topsoil). The 0.6 g (average DM $24 \text{ g} * 0.023$; Food composition table ver. 7) of nitrogen removal by the crop was negligible compared with the input of nitrogen. There was no leaching because there was no rainfall. Finally, the same amount of nitrogen input was lost to the air by denitrification. We used the 3.1 of the K factor (Amato & Ladd, 1988) multiplied for the calculation of SMB-N and non-fumigation nitrogen; however, that overestimated the nitrogen because the total was larger than TN. The K factor could be different, e.g. 2.5, according to the soil (Joergensen & Brookes, 1990). The K factor does not affect the conclusion from the correlation analysis.

Effect of rate of decomposition

The rate of decomposition differed among the different practices. The rate was slow when there was no fertilizer, surface application, and no watering (Table 3). The application of even a small amount of nitrogen remarkably enhanced the rate of decomposition. A strong correlation was found between the change in TN and the change in TC (0.899 in the first half and 0.935 in the second half). The correlation coefficient for the first half increased to 0.959 excluding the 10g nitrogen addition treatment. The change of TN had a -7.79 of negative correlation between in the first half and the latter half. There were no significant correlations between the release (decrease) of nitrogen and SMB ($r = 0.244$ and 0.163 for the first and the latter half respectively).

Discussion

Overview

A conventional yield was achieved under conditions of nitrogen starvation. The order of the yield stably corresponded with the management practice used from season 2. From season 3, SMB increased remarkably; the quantity corresponded with the practice in the similar order as the crop yield. As a result, SMB showed a strong correlation with crop yield from season 3. The applied nitrogen contained in the waste mushroom beds was lost via denitrification, and the rate of decomposition (amount of released nitrogen) showed no correlation with yield or SMB. Accordingly, we concluded 1) the correlation between SMB and crop yield is not a causal relationship, 2) the nitrogen source for both is biological nitrogen fixation.

Fertility as flow

We achieved a conventional yield with a very low $\text{NO}_3\text{-N}$ level. This is inconsistent with nitrogen stock. The same quantity of material was applied each time and the nitrogen equivalent to the applied amount was lost with every crop because TN contents of the initial and the seasonal average were stable, and the change of TC was strongly correlated to that of TN; Namely, no nitrogen from the input remains in the next crop season.

Figure 2. Correlation between yield and SMB-N or TN. ○Season 1, ●Season 2, △Season 3, ▲Season 4 Mean values ($n=4$) for different crop management practices are shown. Season 1 was during the rainy season (water spinach) and the others were during the dry season (lettuce). SMB-N: soil microbial biomass – nitrogen; TN: total nitrogen.

Figure 3. Correlations between SMB-N and yield. Mean values for different crop management practices for seasons 3 and 4 ($n=8$). W: Watering (0: none, 1/w: once per week, 2/d: twice per day); M: Material position (S: surface, A: incorporated, D: incorporated following disinfection by the sun); P: Plant density (0: none, 1: standard, 2: double); N: Nitrogen application (0: none, 0.1: 0.1 g m^{-2} , 10: 10 g m^{-2}); SMB-N: soil microbial biomass – nitrogen.

Table 3. Change in soil TN and TC content ($\mu\text{g g soil}^{-1}$).

Crop management practice		TN				TC			
		Mean		SE		Mean		SE	
		First half	Latter half	First half	Latter half	First half	Latter half	First half	Latter half
N application	0N	164	-63	35	33	-564	-922	444	438
	0.1N	92	14	32	39	-1488	-255	379	452
	10N	143	-2	21	23	-1369	-45	310	331
Plant density	None	148	9	47	53	-966	75	480	538
	Standard	144	-50	21	19	-981	-1107	397	356
	Double	107	-10	18	13	-1474	-190	316	193
Waste mushroom bed	Surface	170	-78	36	28	-651	-1175	526	347
	Dead, mixed	133	-9	19	26	-1221	-462	222	406
	Alive, mixed	95	47	32	32	-1549	415	352	305
Watering	0	153	-66	33	28	-1019	-705	436	289
	1/week	124	-22	14	12	-1224	-590	205	239
	2/day	122	37	41	44	-1178	70	526	613

$n=6$. Change in soil TN and TC in season 4 from the harvesting time of season 3 on days 15 and 38. Waste mushroom bed ($89 \mu\text{g N g soil}^{-1}$ and $1764 \mu\text{g C g soil}^{-1}$) was applied on day 0. First half: day 15 – 0 + Input, Latter half: day 38 – 15. TN: total nitrogen, TC: total carbon.

In addition, the difference in released nitrogen did not affect either crop yield or SMB. These findings led to the conclusion that the stock-based nutrition balance (including TN) is no longer effective under conditions of nitrogen starvation. On the other hand, crop management practices made a remarkable difference. We conclude the treatments make difference in the flow if there is no difference to the stock.

Correlation between SMB and yield

A robust correlation was seen between yield and SMB. However, this was the same response as seen with the different practices. The response was seen in yield from season 2 earlier than the response in SMB from season 3. Therefore, it is unlikely that the nitrogen flow occurs from SMB to crops.

The reason why the SMB suddenly increased from season 3 is unclear. This may have been affected by changes in the soil, such as structural changes due to aggregation, changes in microbial flora, or the accumulation of substances. In the present work, non-fumigation nitrogen increased monotonously.

Nitrogen source of plant and SMB

As mentioned above, applied nitrogen was not utilized by plants or SMB, and the applied nitrogen was lost by denitrification. Despite the denitrification, SMB was still seen to increase considerably in season 3 and 4. This means that SMB obtains nitrogen via biological fixation. Crops also obtain nitrogen by biological fixation because it is unlikely that the nitrogen flow occurs from SMB to crops. This leads to the idea that the application of organic matter with a high C/N ratio enhances the biological nitrogen fixation in the crop root zone by occurring nitrogen starvation, but not providing nitrogen. The amount of the fixed nitrogen is calculated at most 32.1 g m⁻² from the difference of the max and min of SMB in season 3. However, this would be overestimated because the K factor of Amato's may be unsuitable for our experimental conditions. Microbes within the rhizosphere, endosphere, and phyllosphere of plants

interact with crops through numerous mechanisms including nitrogen fixation are gradually clarifying (Compant *et al.*, 2019).

Conclusions

We examined nitrogen flow under conditions of nitrogen starvation through correlation analyses among crops, SMB, and TN. The results showed that the correlation between SMB and crop yield; however, the correlation arises from similar responses to management practices. Namely, the correlation between SMB and crop yield shows no causal relationship. Furthermore, the nitrogen source of both SMB and crop yield are considered biological nitrogen fixation because no correlations were found between the release of nitrogen from applied organic matter and SMB or crop yield. The application of organic matter enhances biological nitrogen fixation by occurring nitrogen starvation but not acting as a nitrogen source. Crop management practices largely affect crop yield. The effect of applying a small amount of nitrogen should be studied to investigate how it can enhance microbial activity. Further study, utilizing 15N natural abundance, will be conducted to determine the source of nitrogen.

Data availability

Underlying data

Figshare: SMB&Yield.xlsb, <https://doi.org/10.6084/m9.figshare.11760963.v1> (Oda & Sukchan, 2020).

Data are available under the terms of the [Creative Commons Zero "No rights reserved" data waiver](#) (CC0 1.0 Public domain dedication).

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Oskar Franklin

International Institute for Applied Systems Analysis, Laxenburg, Austria

The study quantifies the effects of additions of organic matter (SOM) with high C:N ratio, N fertilizer, and water on lettuce crop yield, soil microbial biomass (SMB), and soil N. There is a strong positive correlation observed between SMB and yield. Still it is argued that SOM addition induces N starvation for the crop, and that N does not flow via SMB to crops, which instead get their N from N-fixation.

I am not an expert in this field at all, so I cannot comment on the practical experimental aspects, only on the analysis. While I appreciate concise and short papers, this paper is too short. Many things are not well described, such as the frequency of N application, the significance of the decomposition results, and the definition and estimates of N fixation. These aspects seem to be important for the conclusions so they need to be well explained. Without better explanations I cannot understand the conclusion that SMB is not involved in the provision of N to the crop despite the strong correlation between the two. For example, would it not be possible that the activity of SMB releases inorganic and organic N that is intercepted by plants and is responsible for the yield increase? Rates of microbial N cycling does not necessarily correlate with the microbial biomass.

The estimates of soil microbial N content also seem very high in comparison to total N. In many cases actually higher than total N, which should not be possible.

In conclusion, it is an interesting topic, but this study requires better descriptions of all assumptions and conclusions, and an explanation or re-evaluation of incompatible N values in soil and microbes.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

No

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Process-based ecological modeling and theory

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 06 Jan 2021

Masato Oda, Japan International Research Center for Agricultural Sciences, Tsukuba, Japan

Thank you so much for the precious comments. We made the following improvements.

1. frequency of N application

N application is a starter of decomposition (Added in the “treatments”).

2. the significance of the decomposition results

TC contents of the crop soil were added in the “Fate of waste mushroom bed”.

3. the definition and estimates of N fixation

N fixation is estimated at most 32.1 g m⁻² from the difference of the max and min of SMB in season3 (Added in the “Source of nitrogen”).

4. Would it not be possible that the activity of SMB releases inorganic and organic N that is intercepted by plants and is responsible for the yield increase?

The rate of decomposition (amount of released nitrogen) showed no correlation with yield or SMB.

5. Rates of microbial N cycling does not necessarily correlate with the microbial biomass.

That's right! Our conclusion is that SMB and plant biomass need similar conditions but there are no strong relations between SMB and plant biomass.

6. In many cases actually higher than total N, which should not be possible.

The 3.1 of K factor could be different, e.g. 2.5, according to the soil (Joergensen & Brookes, 1990).

Competing Interests: No competing interests were disclosed.

Reviewer Report 08 September 2020

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Jianwei Li

Department of Agricultural and Environmental Sciences, Tennessee State University, Nashville, TN, USA

The manuscript lacks solid foundation in describing soil nitrogen cycling in general and some of the key nitrogen processes were either ignored or just mentioned without qualification in the studies system. With organic amendments, many biogeochemical processes relevant to nitrogen cycling were not well described. Soil quality is usually improved with organic matter amendments and it may favor nutrient uptake, nitrogen mineralization, diffusion with improved water condition; also nitrogen use efficiency may also increase, that is, crop yield may be higher per unit of nitrogen taken up by the root. I think the current work is too superficial with very poor foundation and justification though it intends to target a very important research question. At the present form, I don't recommend it for indexing.

Is the work clearly and accurately presented and does it cite the current literature?

No

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Soil biogeochemistry

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 14 Sep 2020

Masato Oda, Japan International Research Center for Agricultural Sciences, Tsukuba, Japan

Thank you for considering our manuscript. I guess that you were surprised to see the manuscript.

"Soil quality is usually improved with organic matter amendments and it may favor nutrient uptake, nitrogen mineralization, diffusion with improved water condition."

That's true; however, it is widely known that an organic matter also has negative impacts on plant growth such as nitrogen starvation. Another point of this work is that the work was done in the tropical area of which organic materials are rapidly decomposed. In addition, the soil is sandy soil.

To tell the truth, I was shocked to see the characteristic of the sandy soil in Northeast Thailand for the first.

By the way, what do you think about the data? May I have your comment on the data?

Competing Interests: No competing interests were disclosed.

Comments on this article

Version 1

Reader Comment 05 Mar 2020

Benjamin NOWAK, VetAgro Sup, Lempdes, France

The protocol is not easy to understand, so it is difficult to comment on the results obtained.

Nevertheless, two main remarks can be made:

- It would have been relevant to measure soil nitrogen levels at the beginning of the crop (not just at harvest).
- Fig. 1 is incorrect: you can not use lines to link the different treatments.

Competing Interests: No competing interests were disclosed.

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