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Microbial enzyme activity and stoichiometry signal the effects of agricultural intervention on nutrient cycling in peatlands

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Abstract:

Fertilization in agricultural peatlands accelerates nutrient cycling and creates a potential risk to nearby natural peatlands. Here, using undisturbed peatlands as reference, we studied soil carbon (C), nitrogen (N), phosphorus (P) and the key enzymes for nutrient cycling at 0-50 cm soil depth in agricultural, nearby disturbed peatlands in a temperate fen in Northeast China. Agricultural intervention significantly increased total P in agricultural and disturbed peatlands, and decreased soil organic carbon content and total N in surface soil of agricultural peatlands, however total N significantly accumulated at 20-30 cm soil both in agricultural and disturbed peatlands ($p<0.05$). Both N-acetyl-β-glucosaminidase and phosphatase significantly declined in agricultural peatlands, while only phosphatase decreased in disturbed peatlands ($p<0.05$), and linear regression models showed strong effects of changes of soil nutrient levels on enzyme activities. The ratios of β-D-glucosidase to N-acetyl-β-glucosaminidase and phosphatase markedly increased in agricultural peatlands and showed higher ratios in deeper soil of disturbed peatlands, suggesting relatively higher microbial demand for carbon. Nonmetric multidimensional scaling analysis showed that variations of enzyme activity and stoichiometry can be used to reveal agricultural disturbance, and further redundancy analysis identified that total P and SOC explained 38.3% and 8.3% of the variance. Overall, our findings show that microbial enzymatic activity and stoichiometry can be effective and sensitive indicators of agricultural intervention and nutrient changes in peatlands, which implies that they can be used in monitoring of future fertilization management strategies aimed at fostering more sustainable agriculture.

Key words: fertilization; peatlands; management strategies; nutrient input

Introduction

Peatlands represent a significant atmospheric carbon (C) sink, and hold about 30% of global soil C, despite covering just 3% of the global terrestrial surface (Gorham, 1991). Plant productivity in these ecosystems exceeds decay leading to accumulation over hundreds to thousands of years in the form of increased peat depth and carbon storage (Yu et al., 2010). Peatlands are defined as soils with a high carbon content (>30%) and an organic horizon larger than 30 cm (Rydin and Jeglum, 2006), and in many well developed peatlands, peat depth can exceed two meters (Yu et al., 2010). In recent decades, the need for food and energy, has led to up to 50.9 Mha peatlands being converted to agriculture, grasslands and forestry for food and energy supply (Leifeld and Menichetti, 2018), triggering vast carbon losses (Saurich et al., 2019).

There are about 25 Mha of agricultural peatlands worldwide (Tubiello et al., 2016), representing ～50% of drained peatlands (50.9 Mha) (Leifeld and Menichetti, 2018). Oxygen recovery following anoxic conditions directly promotes microbial metabolic process, turning peatlands from a carbon sink into a hotspot of carbon mineralization (Eickenscheidt et al., 2015; Leifeld and Menichetti, 2018). In addition to oxygen, fertilizer application also contributes to the acceleration of microbial respiration (Eickenscheidt et al., 2015; Saurich et al., 2019). Thus, these all contribute to carbon losses. Bader et al. (2017). reported that organic matter decomposition in agricultural utilized peatlands occurs at a higher rate than either grasslands or peatlands used for forestry.

The additional inorganic nutrients associated with fertilization can easily leach into deeper peat soil (Kogel-Knabner et al., 2010), compounding these effects. Moreover, some studies also show that fertilization increases the nutrient burden of surface and groundwater (Koerselmann et al.,1990; Steinmuller et al., 2016; Berger et al., 2017), potentially leading to eutrophication of nearby pristine peatlands (Wright and Reddy, 2001; Prenger and Reddy, 2004; Steinmuller et al., 2016). Clearly, fertilization is far more detrimental to peatlands than is often assumed, yet few studies have evaluated agricultural fertilization disturbance in pealtands.

Microbial enzyme activities are widely recognized as sensitive indicators of changes in soil function under agricultural management (Lagomarsino et al., 2009; Zagal et al., 2009; Pajares et al., 2009). Soil microorganisms produce extracellular enzymes to acquire nutrients to satisfy the demand for energy for growth, thereby influencing carbon and nutrient cycling (Sinsabaugh and Moorhead, 1994; Luo et al., 2017). Microorganisms also change nutrient acquisition strategies during fertilization (Sinsabaugh and Moorhead, 1994). Although soil physico-chemical properties can reflect the agricultural intervention, short-term changes are usually not easy to detect (Lagomarsino et al., 2009) and fertilization-induced plant composition and biomass changes also influence soil physico-chemical properties (Keller et al., 2006). Peatlands are nutrient-poor systems (Bragazza et al., 2006), and inorganic N and P fertilizer inputs are well known to change microbial enzyme activity and stoichiometry (Pinsonneault et al., 2016; Song et al., 2019). Previous studies have found that agricultural N and P input changed variation in the activities of P and N hydrolase in natural peatlands (Wright and Reddy, 2001; Prenger and Reddy, 2004). While modern agriculture now attempts to create an environment-friendly strategy for sustainable management (Zhang et al., 2012), for agricultural peatlands, maintaining food yields and decreasing fertilizer input in deeper soil itself and its nearby natural peatlands are optimum. Therefore, a full evaluation of the effects of agricultural intervention on microbial enzyme activity and stoichiometry is needed to improve fertilization management in its widest context.

In Northeast China, peatlands have been increasingly cultivated in order to increase food supply since the 1950s, and as cultivated peatlands continue to experience frequent flooding, most were cultivated for rice production. In this study, we investigate the effects of fertilization on both agricultural and nearby natural peatlands in terms of soil carbon (C), nitrogen (N), phosphorus (P) and the key enzymes for C, N and P nutrient cycling. We anticipated (1) that microbial enzymatic activities would be strongly correlated with changes in soil nutrient levels under agricultural intervention and (2) that the variations in microbial enzymatic activity and stoichiometry would exhibit greater similarities in agricultural peatlands than undisturbed peatlands. The information obtained from our study would be important for monitoring nutrient flows from agricultural peatlands and their effects on soil nutrient cycling, potentially greatly improve future strategies for fertilization management.

2 Materials and methods

2.1 Study site

This study was conducted at the Jinchuan Peatlands of Changbai Mountain, Northeastern China. The annual average temperature and precipitation are 4.1 °C and 704.2 mm, respectively. The peat is 4 to 6 m deep in this area and typical plant include *Carex chmidtii*, *Etulao valifolia*, *Phragmites australis* and *Thelypteris palustris*. Peatland reclamation began 1960's mainly for paddy creation. In general, N fertilizer (urea) is applied mid-May, and throughout June for rice growth, totalling 260 kg N ha^{-1} year^{-1}. In contrast, P fertilizer is applied in mid-May only, amounting to 70 kg P ha^{-1} year^{-1}. These agricultural peatlands have been found to influence hydrology and soil organic carbon accumulation of nearby natural peatlands (Zhang et al., 2016; Wang et al., 2017).

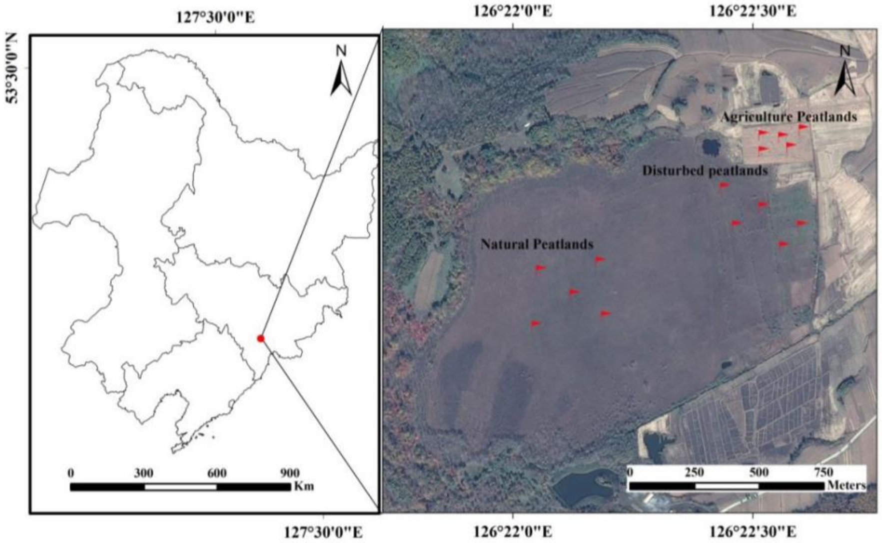

Figure 1 Sampling sites in Jinchuan Peatlands, a temperate fen in Northeastern China

2.2 Sample collection

We selected agricultural peatlands (paddy fields), peatlands disturbed by their close proximity to these agricultural peatlands (disturbed peatlands) and undisturbed peatlands with similar plant composition (Figure 1) as described by Zhang et al. (2016). We established five random sites in each type and distance between each site was at least 20 meters. Five soil cores were collected from each site using a core sampler at 0-10 cm, 10-20 cm, 20-30 cm, 30-40 cm and 40-50 cm soil depths, then each sample from a given depth mixed to make a composite sample for that depth, totally 75 samples in all. Each sample was divided into two parts, one was used for analysis of soil properties and the other for measuring microbial enzyme activity and stored at 4 °C.

2.3 Sample analysis

Soil organic carbon (SOC) was determined by the dichromate oxidation method. Soil total nitrogen (TN) was analyzed using the Kjeldahl method. And soil total phosphorus (TP) was measured by the Mo-Sb colorimetric method.

The determination of microbial enzyme activity was performed as described by Saiya-Cork et al. (2008), which began within one week of sample collection. We selected of β-D-glucosidase (BDG), N-acetyl-β-glucosaminidase (NAG) and phosphatase (PHO) as indicators for C-, N- and P-cycling, respectively (Luo et al., 2017). Briefly, 1.0 g soil was homogenized in 125 ml of acetate buffer (50 mM, pH 5.0) in a blender for 1 min. We conducted assays using 96-well microtiter plates, with eight replicate wells per sample per assay. The analysis included eight replicate wells for each blank (50 μl of acetate buffer plus 200 ul of sample suspension), a negative control (50 μl substrate solution plus 200 μl of acetate buffer), and a quench standard (50 μl of standard 10 mM 4 methylumbelliferone plus 200 μl sample suspension). The microplates were incubated in the dark at 20 °C for 4 h. To stop the reaction, a 10 μl aliquot of 1 M NaOH was added to each well. Fluorescence was measured using a microplate fluorometer with 365 nm excitation and 450 nm emission filters (Synergy H4 BioTek, USA). After correcting for negative controls and quenching, activities were expressed in units of nmol h^{-1} g^{-1} dry soil.

2.4 Statistical analysis

One-way ANOVA was used to determine statistical differences in soil nutrient levels and microbial enzyme activity. Significant differences between means were established by Duncan test at $p<0.05$. The relationship between soil nutrient and microbial enzyme activity were assessed using linear regression model within agricultural, disturbed and undisturbed peatlands. Microbial enzyme activity was log-transformed to meet the assumptions of homoscedasticity. These statistical analyses were performed by SPSS 23.0.

We used Redundancy Analysis (RDA) to know the contribution of environmental variables (SOC, TN, TP and their stoichiometries) to variation of microbial enzyme activity and stoichiometry. Variables were log-transformed and centered to equalize the weight of variables with ranges of different orders of magnitude. Interactive forward selection procedures with unrestricted permutation tests (499 permutations) were used to determine the significant environmental variables to be included in final models. Further, multivariate analysis was performed using two-dimensional nonmetric multidimensional scaling (NMDS) using a Bray–Curtis dissimilarity matrix to calculate the similarities of microbial enzyme activity and stoichiometry among different sites. These statistical analyses were performed using Canoco 5.0 (Microcomputer Power, Ithaca, NY, USA).

3 Results

3.1 The effect of agriculture on soil C, N and P

Compared with undisturbed peatlands, agricultural peatlands SOC content decreased by 29.7 %, 11.8 % and 2.3 % at 0-10 cm, 10-20 cm, and 20-30 cm soil layers ($p<0.05$, Table 1). TN content decreased by 22.4% at 0-10 cm but increased by 6.3 % at 20-30 cm soil depth ($p<0.05$, Table 1). TP content decreased with depth, with the highest 51.7 % at 0-10 cm and lowest 32.7 % in the 40-50 cm soil layer ($p<0.05$, Table 1). In disturbed peatlands, SOC did not show any differences down the profile, TN increased by 7.1 % and 8.3 % at 10-20 cm and 20-30 cm ($p<0.05$, Table 1), respectively. TP increased by 15.9 %, 23.2 %, 26.0 %, and 16.6 % at 0-10 cm, 10-20 cm, 20-30 cm and 30-40 cm, respectively ($p<0.05$, Table 1).

In agricultural peatlands, SOC:TN at 10-30 cm, and both SOC:TN and TN:TP at 0-50 cm were significantly lower than those in undisturbed peatlands ($p<0.05$, Table 1). And in disturbed peatlands, SOC:TN decreased at 10-20 cm, and both SOC:TP and TN:TP at 10-40 cm significantly decreased compared with undisturbed peatlands ($p<0.05$, Table 1).

Table1 The effects of agricultural intervention on soil properties

	Depth (cm)	SOC (g kg^{-1})	TN (g kg^{-1})	TP (g kg^{-1})	SOC:TN	SOC:TP	TN:TP

Agricultural Peatlands	0-10	296.76b	15.07 b	1.12 a	19.62 a	269.11 b	13.56 b
		(30.35)	(1.32)	(0.05)	(0.85)	(34.74)	(1.38)
	10-20	386.86b	19.20 b	1.12 a	20.16 c	348.09 c	17.27 c
		(9.01)	(0.48)	(0.04)	(0.26)	(12.40)	(0.57)
	20-30	423.09b	20.80 a	1.04 a	20.80 b	422.30 c	20.24 c
		(6.2)	(0.27)	(0.05)	(0.56)	(24.48)	(0.80)
	30-40	437.54 a	20.77 a	0.97 a	21.13 a	453.74 c	21.51 c
		(7.93)	(0.54)	(0.03)	(0.70)	(19.75)	(0.86)
	40-50	431.76b	20.21 a	0.96 a	21.39 a	450.18 b	21.02 b
		(8.29)	(0.40)	(0.04)	(0.47)	(23.26)	(0.82)
Disturbed Peatlands	0-10	426.47 a	20.14 a	0.86 b	21.28 a	497.68 a	23.50 a
		(7.10)	(0.76)	(0.01)	(0.78)	(15.93)	(1.06)
	10-20	4443.79 a	20.66 a	0.90 b	21.48 b	493.76 b	22.99 b
		(4.01)	(0.0.20)	(0.02)	(0.14)	(13.74)	(0.64)
	20-30	455.08 a	21.19 a	0.83 b	21.51 ab	549.01 b	25.57 b
		(3.89)	(0.38)	(0.01)	(0.53)	(5.68)	(0.55)
	30-40	451.28 a	19.55 a	0.78b	23.18 a	580.23 b	25.12 b
		(8.36)	(0.83)	(0.01)	(0.68)	(11.06)	(0.95)
	40-50	453.93 a	20.68 a	0.79 b	22.06 a	574.88 a	26.14 a
		(4.49)	(0.77)	(0.02)	(0.77)	(10.89)	(0.68)
Undisturbed Peatlands	0-10	422.10 a	19.41 a	0.74 c	21.79 a	588.52 a	26.25 a
		(6.05)	(0.35)	(0.01)	(0.61)	(23.92)	(0.65)
	10-20	438.44 a	19.30 b	0.73 c	22.74 a	600.94 a	26.43 a
		(3.17)	(0.25)	(0.02)	(0.46)	(17.70)	(0.53)
	20-30	442.44 ab	19.57 b	0.66 c	22.63 a	672.57 a	29.74 a
		(5.55)	(0.30)	(0.01)	(0.20)	(6.48)	(0.48)
	30-40	439.89 a	18.87 a	0.67 c	22.27 a	660.98 a	28.47 a
		(5.55)	(0.52)	(0.02)	(0.81)	(22.58)	(0.95)
	40-50	444.63 ab	19.22 a	0.73 b	23.21a	613.35 a	26.44 a
		(6.67)	(0.45)	(0.02)	(0.84)	(24.51)	(0.57)

Notes: Different letters indicate significant differences at the same depth. Soil organic carbon (SOC); Total nitrogen (TN); Total phosphorus (TP).

3.2 The effect of agricultural intervention on microbial enzymatic activities

In undisturbed peatlands, BDG and PHO activity showed high similarity at 0-50 cm depth (Figure 2a & c). Compared with undisturbed peatlands, in agricultural peatlands, BDG activity did not show any differences, although NAG and PHO activity significantly decreased across the depths (Figure 2b). In disturbed peatlands, BDG activity significantly increased at 0-10 cm and 30-40 cm, NAG activity significantly increased at 0-10 cm and decreased at 20-30 cm and 40-50 cm, and PHO activity significantly declined at all the depths.

In agricultural and disturbed peatlands, both BDG:NAG and BDG:PHO were significantly higher at all the depth than those in undisturbed peatlands ($p<0.05$, Figure 3a &

b). Overall, they also showed higher ratios in agricultural peatlands than disturbed peatlands (Figure 3a & b).

Figure 2 The effects of agricultural intervention on microbial enzyme activities. (a) β-D-glucosidase (BDG); (b) N-acetyl-β-glucosaminidase (NAG); (c) phosphatase (PHO). Different letters indicated significant differences at same depth.

Figure 3 The effects of agricultural intervention on microbial enzyme stoichiometry. (a) ratio of β-D-glucosidase to N-acetyl-β-glucosaminidase (BDG:NAG); (b) ratio of β-D-glucosidase to phosphatase (BDG:PHO); (c) ratio of N-acetyl-β-glucosaminidase to phosphatase (NAG:PHO). Different letters indicated significant differences at the same depth.

3.3 Correlation between soil nutrients and microbial enzyme activities

Linear regression model showed that SOC significantly increased BDG, NAG and PHO activity, respectively ($p<0.05$, Figure 4adg). TN was positively correlated with BDG and NAG activity, respectively ($p<0.05$, Figure 4b&e). And TP significantly decreased NAG and PHO activity, respectively ($p<0.01$, Figure 4f&i).

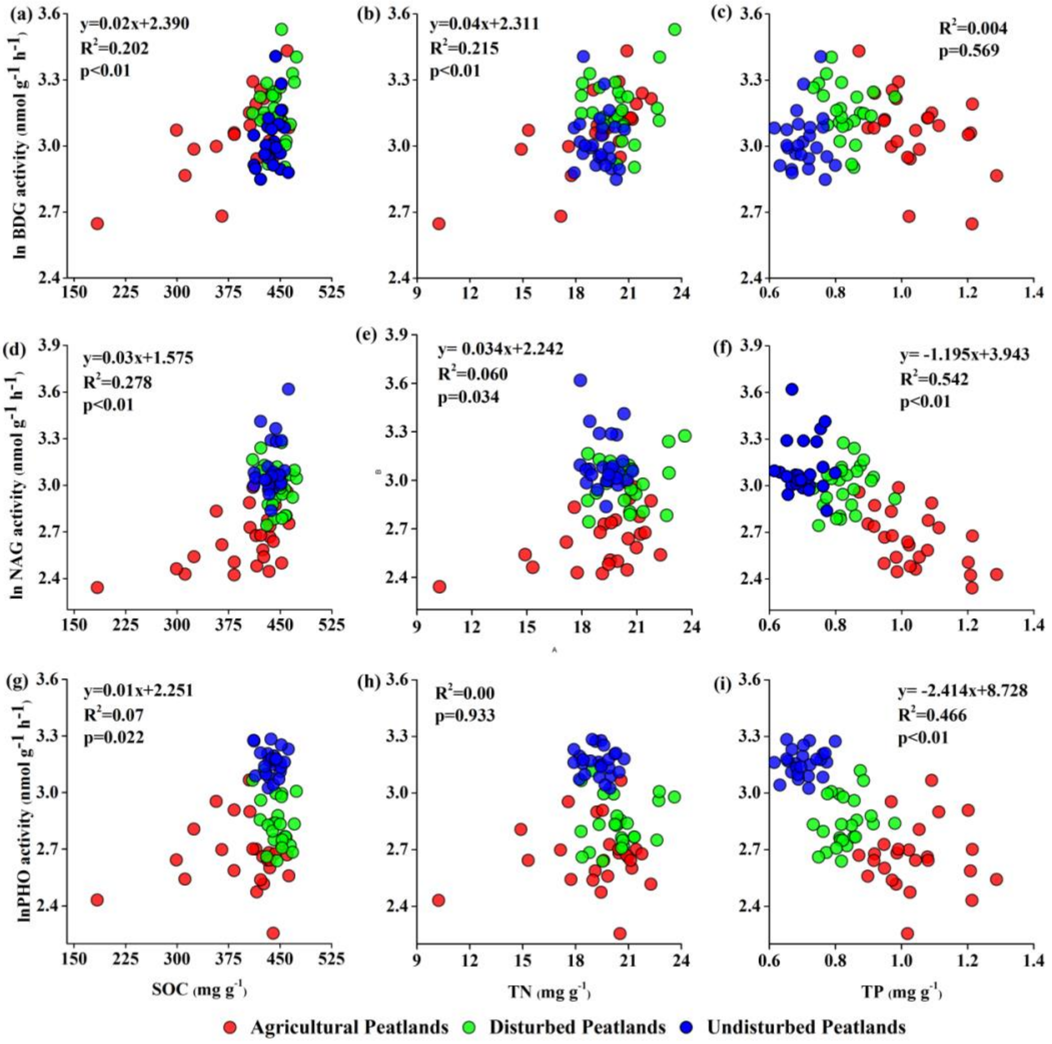

Figure 4 The relationship between soil nutrient and microbial enzyme activity. β-D-glucosidase (BDG); N-acetyl-β-glucosaminidase (NAG); phosphatase (PHO); Soil organic carbon (SOC); total nitrogen (TN); total phosphorus (TP).

3.4 Influences of agricultural intervention on microbial enzyme activity and stoichiometry

Clusters in enzymatic activity and stoichiometry in agricultural, disturbed and undisturbed peatlands can be seen in the NMDS ordination graph (Figure 5). Variations in agricultural and disturbed peatlands showed a degree of similarity, which was absent in that of undisturbed peatlands.

The SOC, TN, TP and their stoichiometries explain 50.8% of the variation of microbial enzymatic activity and their stoichiometry based on ordination analysis using redundancy analysis (Table 2 & Figure 6). And TP and SOC were determined to be factors significantly explaining 38.3% and 8%, respectively (Table 2 & Figure 6).

Figure 5 NMDS analysis of the composition of microbial enzyme activity and stoichiometry. Squares represent agricultural peatlands, circles represent disturbed peatlands, triangles represent undisturbed peatlands. Yellow, red, green, blue, and black represent 0-10 cm, 10-20 cm, 20-30 cm, 30-40 cm and 40-50 cm, respectively. Resemblance distance measure: Bray-Curtis similarity.

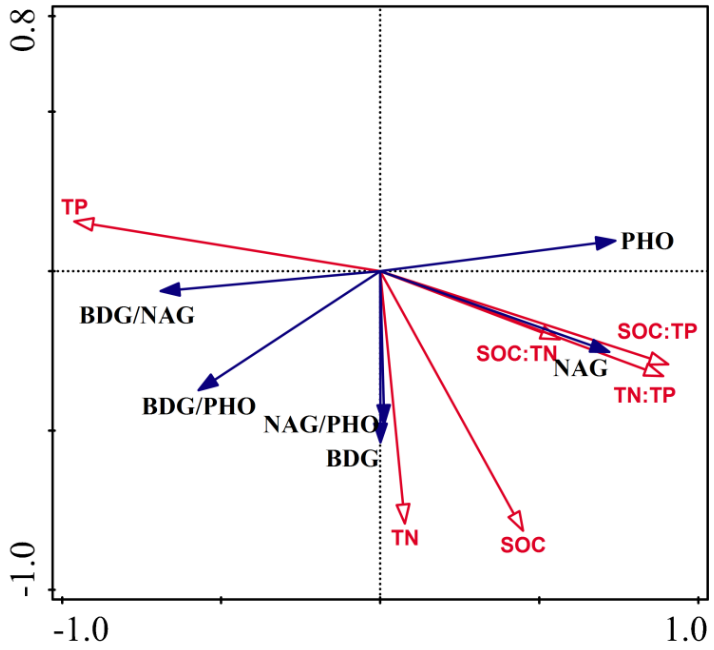

Figure 6 Redundancy analysis ordination plot of enzymatic variables constrained by environmental variables. Enzymatic data were log-transformed and centered to normalize weights of data due to differences in orders of magnitude and ranges.

Table 2 Results of redundancy analysis model of enzymatic variation using environmental variables, determined by forward selection procedure with unrestricted permutation tests the contribution of variables. Soil organic carbon (SOC); Total nitrogen (TN); Total phosphorus (TP).

Variables	Explain(%)	Contribution(%)	Pseudo F	p
TP	38.3	75.5	45.3	0.002
SOC	8.0	15.8	10.7	0.002

TN	0.8	1.5	1.1	0.358
SOC:TN	1.7	3.3	2.3	0.354
SOC:TP	1.2	2.3	1.6	0.358
TN:TP	0.8	1.6	1.1	0.354
Total	50.8	100		

4 Discussion

4.1 Soil C, N and P changes associated with contrasting levels of agricultural influence

Agricultural activities have been found to influence hydrology in peatlands, which increases surface peat aerobic decomposition in agricultural and disturbed peatlands (Zhang et al., 2016; Wang et al., 2017). Peatlands are widely acknowledged as N or P limited ecosystem (Hill et al., 2012), and as such, any increase in nutrient availability potentially stimulates organic matter decomposition, and causes fertilization-induced plant biomass changes (Keller et al., 2006), which further influences SOC. These could best explain the large soil organic carbon loss in surface soil of agricultural peatlands and weak variance in disturbed peatlands.

Inorganic fertilization would be expected to cause nutrients to leach deeper into a given soil or from agricultural peatlands to nearby natural peatlands, changing soil nutrient levels therein (Kogel-Knabner et al., 2010; Steinmuller et al., 2016). In our study we found that in agricultural peatlands, TN was 22.4% lower in surface 0-10 cm soil than in less disturbed soils suggesting a higher degree of N mobility, and losses potentially through leaching. In the nearby peatlands, TN remained unchanged at the surface 0-10cm but increased 7.3-8.3 % at 10-30 cm depth (Table 1). Total P in agricultural peatlands was higher than in disturbed peatlands, especially in the surface soil (Table 1). Previous studies have shown N or P fertilizer uptake by crops and harvest can reduce nutrient abundance in surface soils (Cao et al., 1984), however, plant-assimilated N and P would return to the soil through plant decomposition in natural peatlands. It is likely that this is observed because reactive nitrogen is easily leached (Kogel-Knabner et al., 2010) and liable to oxidation through nitrification, denitrification and feammox (Yang et al., 2012; Shi et al., 2017), all contributing to N loss in these upper layers. In contrast, P is easily absorbed by soil minerals (Zhao et al., 2018), and previous studies show that surface soils in this area contain an abundance of minerals (Qin et al., 2020), which would help to retain P surface soil of these agricultural peatlands. However, many factors influence soil nutrient levels, and overall there is little doubt that the observed increased N and P levels indicate that fertilization affects soil nutrient levels in agricultural peatlands and then further impacts neighboring peatlands. Soil C:N has been suggested as an indicator for nutrient transformation (Spohn et al., 2013; Hu et al., 2019), in our study, there were no differences in C:N at 30-50 cm in agricultural or disturbed peatlands compared with undisturbed peatlands, providing reassurance that there are limits to the depths affected by fertilizer application. This is most likely due to the low hydraulic conductivity at depth in peatlands.

4.2 The response of microbial enzymatic activity to agricultural intervention

Compared soil physico-chemical properties, microbial enzymatic activities may provide an earlier warning of the effects of agricultural intervention by reflecting changes in C, N and P levels as they begin to occur (Gil-Sotres et al., 2005; Lagomarsino et al., 2009; Burns et al., 2013). In our study, both NAG and PHO activity significantly decreased down the profile in agricultural peatlands, a trend also seen at least for PHO activity, in the more disturbed peatlands. Ratios of BDG to NAG and BDG to PHO significantly increased down the profile in agricultural peatlands and deeper soil in disturbed peatlands, suggesting greater microbial demand for carbon (Luo et al., 2017). Further regression analysis showed that SOC positively influence BDG, NAG and PHO activity, while total N positively influenced BDG and PHO activity, however, total P negatively influenced BDG and PHO activity ($p<0.05$, Figure 4b & e). According to the resource allocation models, increased inorganic N or P availability could decrease microbial N and P acquiring enzyme activities leading to increases in of microbial C acquisition (Sinsabaugh and Moorheas, 1994; Pinsonneault et al., 2016). C and N are essential substrates and nutrients for microorganisms to be able to synthesize enzymes and support productivity (Olander and Vitousek, 2000).

However, as proteins, phosphatases have relatively high N concentrations (between 8% and 32%), may represent a significant investment of N (Treseder and Vitousek, 2001), and high P may also decrease microbial N demand. Furthermore, microbial growth and function could also be inhibited in presence of excessive P (Conrad et al., 2000; Li et al., 2017). Based on the degree of correlation, P strongly inhibited NAG activity despite its promotion by SOC and total N (Figure 4).

Clearly the microbial enzyme activities and their respective stoichiometries sensitively reflect the agricultural intervention based on NMDS. Moreover, RDA analysis confirmed that total P and SOC were determining factors that significantly explain 38.3% and 8% of the variation, respectively. These observations confirm that microbial enzymatic activity and stoichiometry are strongly influenced by changes of nutrient levels and agricultural intervention (Lagomarsino et al., 2009; Jian et al., 2016).

Implications for managements in agricultural peatlands

N fertilizer consumption has increased by 18% over a period of just 20 years as part of global efforts to increase crop yields (Allen and Beatty, 2011). Generally, N fertilizers are over-applied, at rates far exceeding the maximum demand of the crop (Allen and Beatty, 2011). Not only is this a waste of resources but it also results in nitrogen pollution of the atmosphere, of rivers and of the oceans. Nutrient inputs also destabilize carbon stores by increasing organic matter decomposition, and thereby indirectly decreases soil N retention capacity (Zhu and Wang et al., 2011). This can mislead farmers into believing that crops need even more N fertilization with long-term tillage, further exacerbating N fertilization rates. P is easily absorbed by mineral (Emsens et al., 2017), leaching and runoff with water is the main P loss pathways, from this point, P fertilization seems much abundant than N fertilizer in such agricultural system, it is necessary to reduce the quantity of P fertilizer.

Modern agricultural practice now sees integrated nutrient management as the most sustainable strategy for increasing food production as this decreases chemical fertilizer consumption (Zhang et al., 2012). Many studies have focused on crop nutrient uptake, nutrient supply in root zone, and fertilization loss (Zhang et al., 2012; Yousaf et al., 2016). However, the environment peat occurs high water table levels and soils with high hydraulic conductivity near the surface that favor lateral water movement. Agricultural activity in such peatlands can as a consequence impart wider effects on nearby natural pealtands as a result of the connectivity through surface and groundwater flows. Thus, monitoring and evaluation is important for maintaining natural peatland system stability and ecological services. Our study, suggests that soil microbial enzymatic activity and stoichiometry could provide an effective early warning indicator for risks from agricultural peatlands to their adjacent natural systems. Moreover, coupled analyses of soil properties and microbial enzyme activities could provide a detailed insight into soil carbon and nutrient cycling, which can ensure that the risks from fertilizer management in agricultural peatlands towards their adjacent natural ecosystems is minimized.

Conclusions:

Compared with undisturbed peatlands, soil properties in agricultural and disturbed peatlands showed significant impacts from disturbance that were strongly correlated with changes in microbial enzymatic activities. When coupled with consequent changes in microbial enzymatic stoichiometry and changes detected soil nutrient levels, far more sensitive indicators of ecological changes are achievable than by measuring soil properties alone. Variations in microbial enzymatic activity and stoichiometry proved to be highly responsive to agricultural intervention. Thus, such measures are proposed as valuable indicators of agricultural intervention that could be of great value in monitoring the success of future fertilization strategies aimed at a more sustainable approach to agriculture.

Acknowledgments

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Figures & Tables Captions:

Figure 1 Sampling sites in Jinchuan Peatlands, a temperate fen in Northeastern China.

Figure 2 The effects of agricultural intervention on microbial enzyme activities. (a) β-D-glucosidase (BDG); (b) N-acetyl-β-glucosaminidase (NAG); (c) phosphatase (PHO). Different letters indicated significant differences at same depth.

Figure 3 The effects of agricultural intervention on microbial enzyme stoichiometry. (a) ratio of β-D-glucosidase to N-acetyl-β-glucosaminidase (BDG:NAG); (b) ratio of β-D-glucosidase to phosphatase (BDG:PHO); (c) ratio of N-acetyl-β-glucosaminidase to phosphatase (NAG:PHO). Different letters indicated significant differences at same depth.

Figure 4 The relationship between soil nutrient and microbial enzyme activity. β-D-glucosidase (BDG); N-acetyl-β-glucosaminidase (NAG); phosphatase (PHO); Soil organic carbon (SOC); total nitrogen (TN); total phosphorus (TP).

Figure 5 NMDS analysis of the composition of microbial enzyme activity and stoichiometry. Squares represent agricultural peatlands, circles represent disturbed peatlands, triangles represent undisturbed peatlands. Yellow, red, green, blue, and black represent 0-10 cm, 10-20 cm, 20-30 cm, 30-40 cm and 40-50 cm, respectively. Resemblance distance measure: Bray-Curtis.

Figure 6 Redundancy analysis ordination plot of enzymatic variables constrained by environmental variables. Enzymatic data were log-transformed and centered to normalize weights of data due to differences in orders of magnitude and ranges.

Table1 The effects of agriculture intervention on soil properties. Different letters indicate significant differences at same depth. Soil organic carbon (SOC); Total nitrogen (TN); Total phosphorus (TP).

Table 2 Results of redundancy analysis model of enzymatic variation using environmental variables, determined by forward selection procedure with unrestricted permutation tests the contribution of variables.

Figure 1

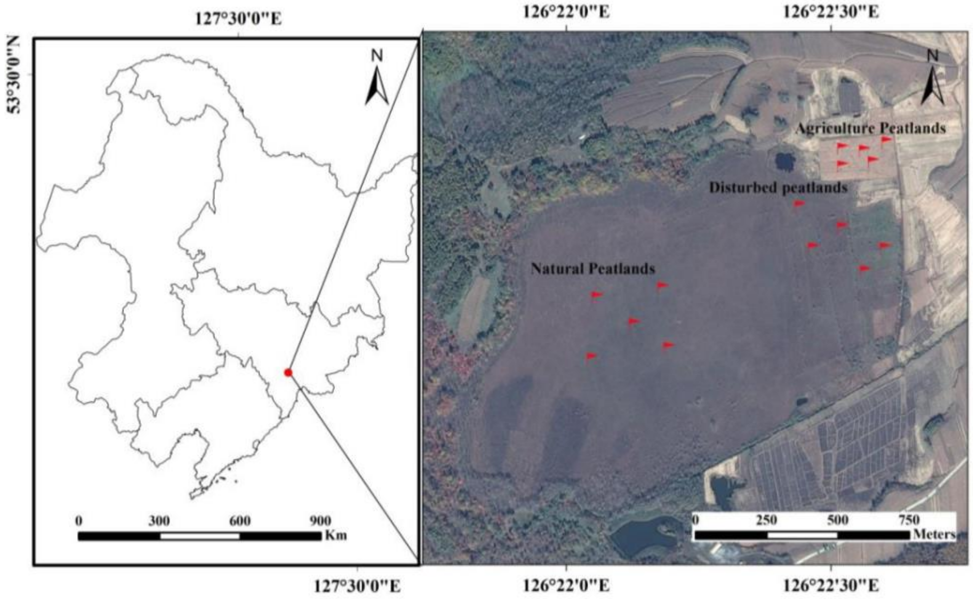


Figure 2

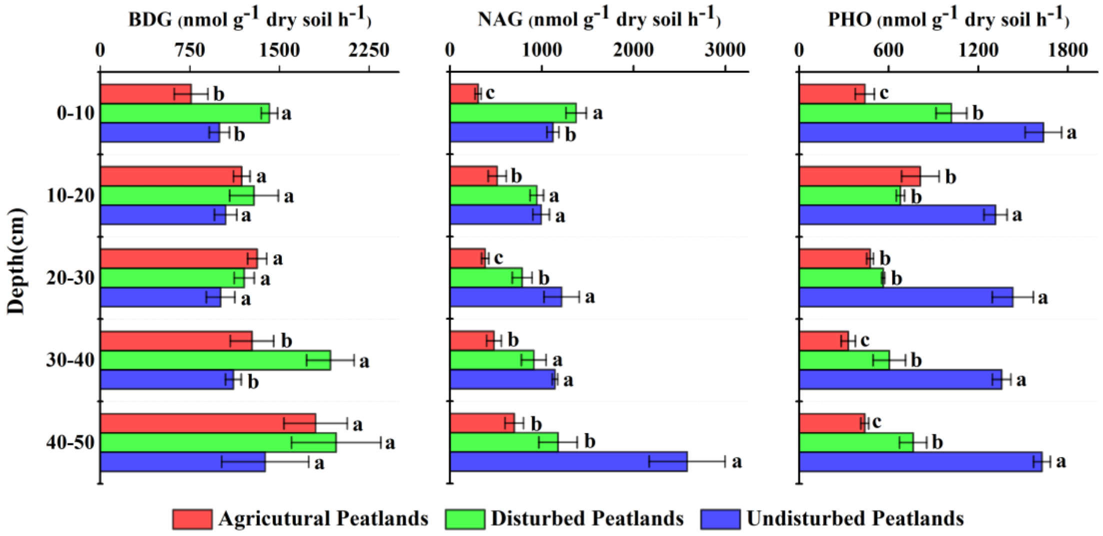

BDG (nmol g-1 dry soil h-1)
0
750
1500
2250
NAG (nmol g-1 dry soil h-1)
0
1000
2000
3000
PHO (nmol g-1 dry soil h-1)
0
600
1200
1800
Depth(cm)
0-10
10-20
20-30
30-40
40-50
b
a
b
a
a
a
a
a
a
b
a
b
a
a
a
c
a
b
b
a
a
c
b
a
b
a
a
b
b
a
c
b
a
b
b
a
b
b
a
c
b
a
c
b
a
Agricutural Peatlands
Disturbed Peatlands
Undisturbed Peatlands

Figure 3

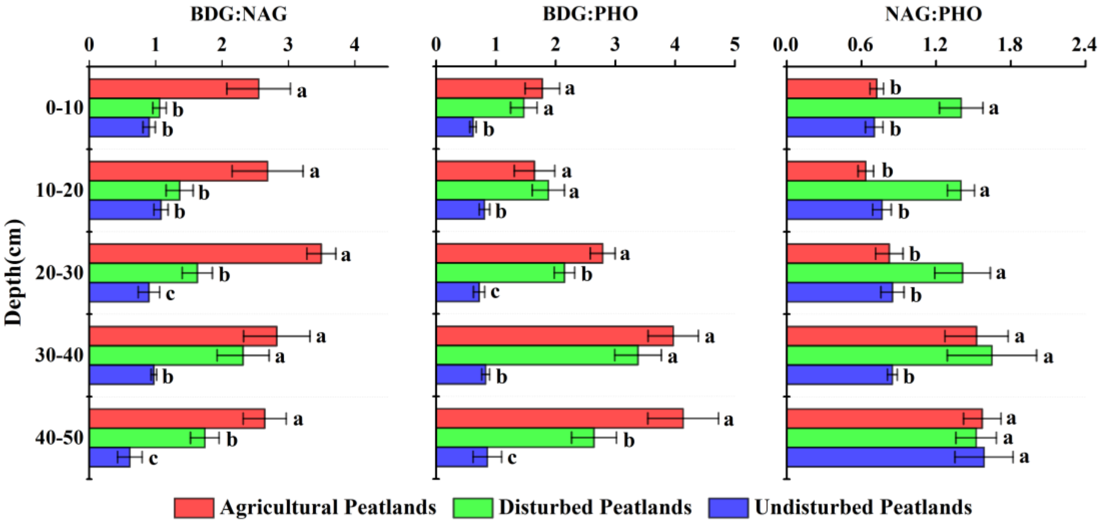

BDG:NAG
BDG:PHO
NAG:PHO
0
1
2
3
4
5
0.0
0.6
1.2
1.8
2.4
Depth(cm)
0-10
10-20
20-30
30-40
40-50
a
b
c
Agricultural Peatlands
Disturbed Peatlands
Undisturbed Peatlands

Figure 4

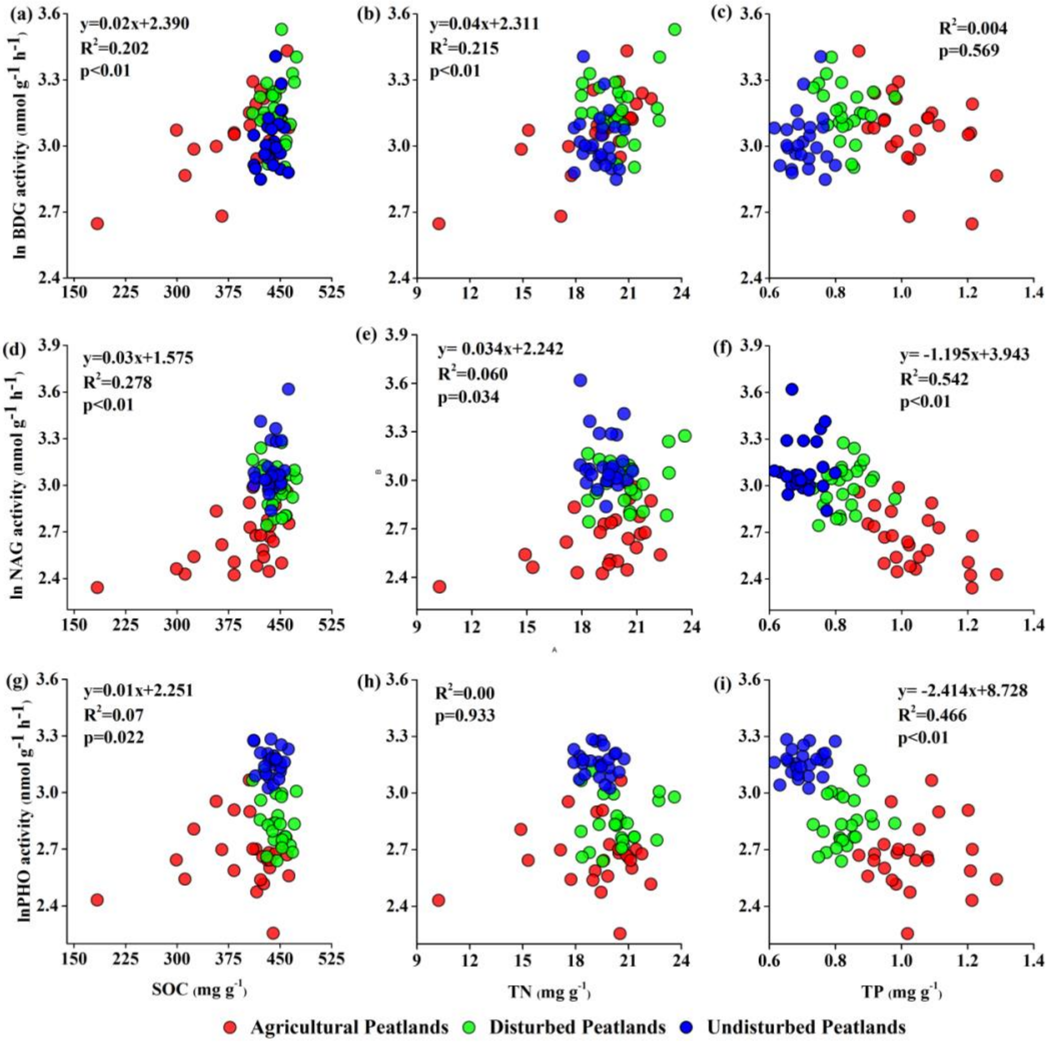
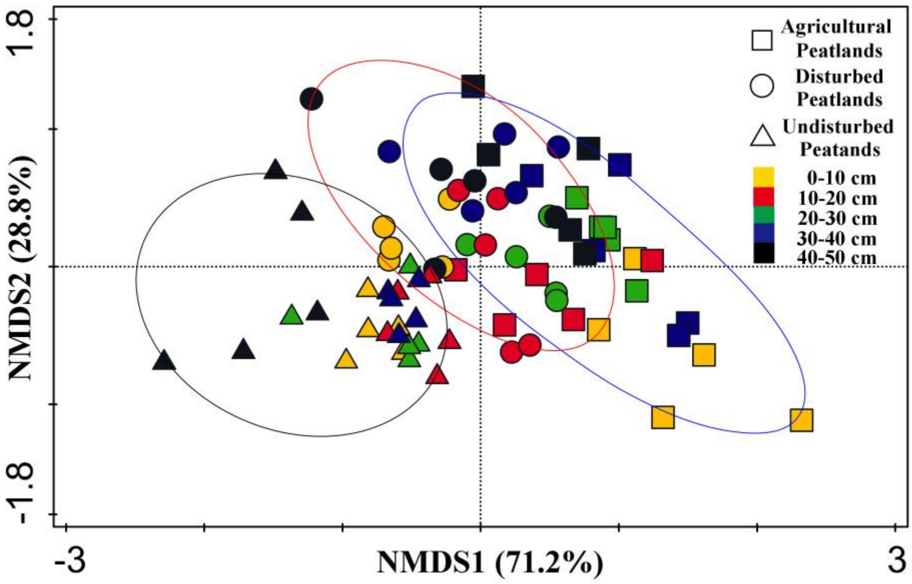

(a)
y=0.02x+2.390
R²=0.202
p<0.01
(b)
y=0.04x+2.311
R²=0.215
p<0.01
(c)
R²=0.004
p=0.569
ln BDG activity (nmol g-1 h-1)
(d)
y=0.03x+1.575
R²=0.278
p<0.01
(e)
y= 0.034x+2.242
R²=0.060
p=0.034
(f)
y= -1.195x+3.943
R²=0.542
p<0.01
ln NAG activity (nmol g-1 h-1)
(g)
y=0.01x+2.251
R²=0.07
p=0.022
(h)
R²=0.00
p=0.933
(i)
y= -2.414x+8.728
R²=0.466
p<0.01
lnPHO activity (nmol g-1 h-1)
SOC (mg g-1)
TN (mg g-1)
TP (mg g-1)
150 225 300 375 450 525
9 12 15 18 21 24
0.6 0.8 1.0 1.2 1.4
2.4 2.7 3.0 3.3 3.6 3.9
Agricultural Peatlands
Disturbed Peatlands
Undisturbed Peatlands

Figure 5


1.8
-1.8
NMDS2 (28.8%)
-3
3
NMDS1 (71.2%)
Agricultural Peatlands
Disturbed Peatlands
Undisturbed Peatands
0-10 cm
10-20 cm
20-30 cm
30-40 cm
40-50 cm

similarity.

Figure 6

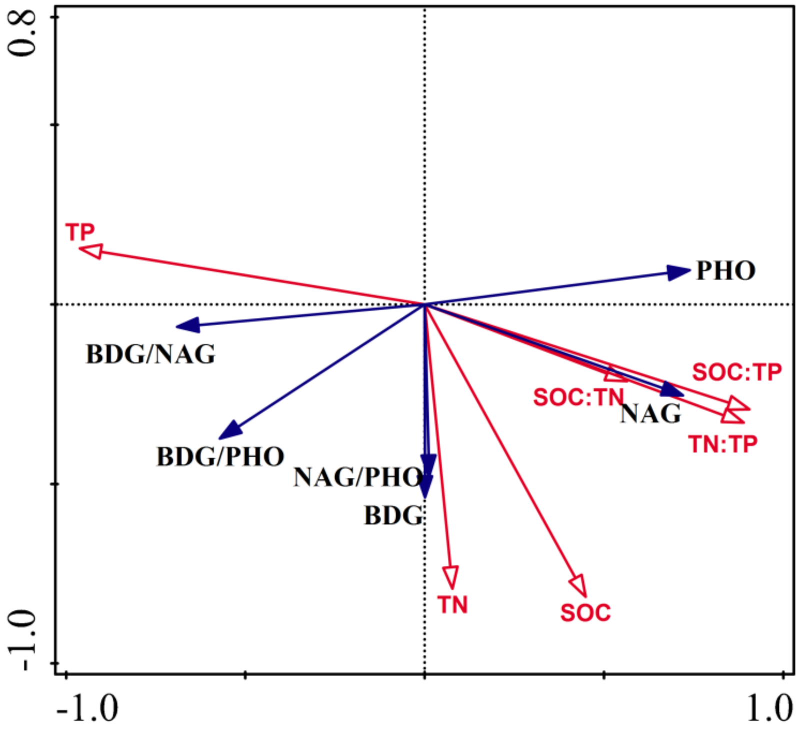

0.8
-1.0
-1.0
1.0
TP
PHO
BDG/NAG
SOC:TP
SOC:TN
NAG
TN:TP
BDG/PHO
NAG/PHO
BDG
TN
SOC

Table1

Table1 The effects of agricultural intervention on soil properties

	Depth (cm)	SOC (g kg^{-1})	TN (g kg^{-1})	TP (g kg^{-1})	SOC:TN	SOC:TP	TN:TP
Agricultural Peatlands	0-10	296.76b (30.35)	15.07 b (1.32)	1.12 a (0.05)	19.62 a (0.85)	269.11 b (34.74)	13.56 b (1.38)
	10-20	386.86b (9.01)	19.20 b (0.48)	1.12 a (0.04)	20.16 c (0.26)	348.09 c (12.40)	17.27 c (0.57)
	20-30	423.09b (6.2)	20.80 a (0.27)	1.04 a (0.05)	20.80 b (0.56)	422.30 c (24.48)	20.24 c (0.80)
	30-40	437.54 a (7.93)	20.77 a (0.54)	0.97 a (0.03)	21.13 a (0.70)	453.74 c (19.75)	21.51 c (0.86)
	40-50	431.76b (8.29)	20.21 a (0.40)	0.96 a (0.04)	21.39 a (0.47)	450.18 b (23.26)	21.02 b (0.82)
Disturbed Peatlands	0-10	426.47 a (7.10)	20.14 a (0.76)	0.86 b (0.01)	21.28 a (0.78)	497.68 a (15.93)	23.50 a (1.06)
	10-20	4443.79 a (4.01)	20.66 a (0.0.20)	0.90 b (0.02)	21.48 b (0.14)	493.76 b (13.74)	22.99 b (0.64)
	20-30	455.08 a (3.89)	21.19 a (0.38)	0.83 b (0.01)	21.51 ab (0.53)	549.01 b (5.68)	25.57 b (0.55)
	30-40	451.28 a (8.36)	19.55 a (0.83)	0.78b (0.01)	23.18 a (0.68)	580.23 b (11.06)	25.12 b (0.95)
	40-50	453.93 a (4.49)	20.68 a (0.77)	0.79 b (0.02)	22.06 a (0.77)	574.88 a (10.89)	26.14 a (0.68)
Undisturbed Peatlands	0-10	422.10 a (6.05)	19.41 a (0.35)	0.74 c (0.01)	21.79 a (0.61)	588.52 a (23.92)	26.25 a (0.65)
	10-20	438.44 a (3.17)	19.30 b (0.25)	0.73 c (0.02)	22.74 a (0.46)	600.94 a (17.70)	26.43 a (0.53)
	20-30	442.44 ab (5.55)	19.57 b (0.30)	0.66 c (0.01)	22.63 a (0.20)	672.57 a (6.48)	29.74 a (0.48)
	30-40	439.89 a (5.55)	18.87 a (0.52)	0.67 c (0.02)	22.27 a (0.81)	660.98 a (22.58)	28.47 a (0.95)
	40-50	444.63 ab (6.67)	19.22 a (0.45)	0.73 b (0.02)	23.21a (0.84)	613.35 a (24.51)	26.44 a (0.57)

Notes: Different letters indicate significant differences at the same depth. Soil organic carbon (SOC); Total nitrogen (TN); Total phosphorus (TP).

Table 2 Results of redundancy analysis model of enzymatic variation using environmental variables, determined by forward selection procedure with unrestricted permutation tests the contribution of variables. Soil organic carbon (SOC); Total nitrogen (TN); Total phosphorus (TP).

Variables	Explain(%)	Contribution(%)	Pseudo F	p
TP	38.3	75.5	45.3	0.002
SOC	8.0	15.8	10.7	0.002
TN	0.8	1.5	1.1	0.358
SOC:TN	1.7	3.3	2.3	0.354
SOC:TP	1.2	2.3	1.6	0.358
TN:TP	0.8	1.6	1.1	0.354
Total	50.8	100		