Potato Fermented Fertilizer Modulates Soil Nitrification by Shift Niche of Functional Microorganisms to Increase Yield in North China

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Research Article

Keywords: Potato Fermented Fertilizer, Soil microbial community, Comammox, AOA, AOB

Posted Date: February 17th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1341972/v1

Abstract

Aims

Potato starch wastewater contains higher contents of essential nutrients, which can be feritilizer to help crop growth. However, the effects of potato fermented fertilizer on soil ecology and soil microbial community structure have not yet been elucidated. The objective of this study was to investigate the shifts of active ammonia oxidation microbial communities under different fertilization in a typical soil from North China.

Methods

The different levels of potato fermented fertilizer without or with chemical fertilizer were designed by field experiment.

Results

The results showed that the application of potato fermented fertilizer could significantly increase crop yields by 165–399% compared to Control. The content of available soil nutrients and the activity of saccharase and cellulase were increased when potato fermented fertilizer was applied, and the combination fertilizers further increased the content of Olsen-P by 145.6-166.7%, NO_3^- by 15.2–81.1%, Total C by 13.8–14%, and Total N by 27.2–34.7% compared with potato fermented fertilizer (PW) treatments. Furthermore, the fermented potato fertilizer significantly stimulated the diversity of soil microbial community, and increased the differentiation and stability of soil microbial networks in deep soils. Finally, the change of niche of soil Comammox(COM), ammonia-oxidizing archaea (AOA), and ammonia-oxidizing bacteria (AOB) was found after PW treatments, showed a significant positive correlation between AOA and COM ($r = 0.79$, $P < 0.01$), AOB and NOB ($r = 0.7$, $P < 0.05$) instead of theoretically the competitive relationship between AOA and COM.

Conclusions

Potato fermented fertilizer modulates soil nitrification strategy by change the niche of soil functional microorganisms to increase fast-acting nutrients and increase crop yield.

Introduction

Potato (*Solanum tuberosum L.*), a crop with high nutritional and industrial values, is cultivated on massive agric soils worldwide. Potato is the largest non-cereal food crop worldwide and is the fourth most important food crop after wheat, corn, and rice. About 50% of all cultivated potatoes are used in processing industries such as potato starch and feed processing industries (Wang et al., 2009). In the potato starch industry, it has been estimated that 7 m^3 of potato wastewater is produced during the processing of 1-ton potatoes. Subsequently, the potato wastewaters are treated through hot coagulation to remove about 90% of the protein to get deproteinized potato wastewater (Miedzianka et al., 2014).

The potential application of deproteinized potato wastewater in the field has numerous benefits. Firstly, potato waste contains abundant protein and amino acids. Notably, the decomposition of amino acids into more minor soluble compounds is critical in the terrestrial nitrogen cycle because it can provide an abundant source of nitrogen compounds and mineral substances for soil microorganisms (Noll et al., 2019). Moreover, potato wastewater contains significant amounts of mineral compounds (approximately 1%), dominated by potassium and phosphorus (Kot et al., 2020). Over

the years, studies have been conducted to assess the effects of potato wastewater as fertilizer on soil nutrients and crop yields, but has not been done the effects of potato wastewater as fertilizer on soil nutrient transformation and soil functional microorganisms.

Nitrogen (N) is one of the essential nutrients for plants, and soil microorganisms can transform soil N into a usable form for plants through the catalytic nitrification process. Nitrification was long considered a two-step process involving ammonia to nitrite, followed by oxidation of nitrite to nitrate. It is worth noting that ammonia oxidation is catalyzed by ammonia-oxidizing microorganisms, including ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) (Nunes-Alves, 2016; Abbas et al., 2020). Since the discovery of AOA, the contribution of AOA and AOB to ammonia oxidation and the ecological niches of AOA and AOB have been the research hotspots. However, to date, there is still controversy about the metabolic mode of AOA. The traditional view is that both AOA species are strictly autotrophic microorganisms (Kim et al., 2016). However, it has been widely documented that organic carbon is an important environmental factor affecting the AOA community. For example, the abundance of AOA still increased when the nitrification activity was completely inhibited by acetylene, suggesting that AOA may have heterotrophic activity (Prosser and Nicol, 2008; Jia and Conrad, 2009). Liu et al. (2018) also revealed that long-term manure application increased AOA abundance in some soils. The recent discovery of comammox bacteria (complete ammonia oxidizers) has also challenged the conventional theory of two-step nitrification (Daims et al., 2015a; Van Kessel et al., 2015). The high frequency of comammox bacteria was later reported in various artificial and natural habitats such as soil, rivers, sediments, drinking water treatment plants, and a range of wastewater treatment bioreactors, which suggests that comammox bacteria play a vital role in the N cycle (Cotto et al., 2020). The discovery of comammox bacteria led to a series of questions: (i) how is comammox regulated by environmental factors (ii) Is there niche differentiation of comammox, AOA, and AOB, and (iii) How does the discovery of comammox change our perception of the global N cycle (Santoro, 2016). The answer to the third question is based on the previous two questions. Currently, several studies have been conducted to answer the first question. For example, Liu et al. (2020b) found that comammox *Nitrospira*, including clade A and clade B, contributed more to nitrifiers abundance in typical oligotrophic environments with a higher pH and lower temperature, particularly clade A in the plateau and clade B in mountain and foothill areas of the upper reach. In addition, Xu et al. (2020) reported that the growth of comammox *Nitrospira* favors slightly alkaline soil with relatively high C/N and low ammonia conditions. However, there are only a few studies on the respective roles of AOA, AOB, and comammox in nitrification and their association with each other under organic and inorganic fertilizers.

The objective of this study was to investigate the shifts of active ammonia oxidation microbial communities under different fertilization in a typical soil from North China by field experiments. Moreover, we investigated the effects of different levels applications of fermented potato fertilizer without or with chemical fertilizer on crop yield, soil physicochemical properties, enzyme activity, and niche differentiation of comammox, AOA, and AOB

Materials And Methods

Experimental site

The experiment was conducted in Pingjibao Experimental Greenhouse, Guyuan, Ningxia, China (N 35° 49' 54", E 106° 40' 29") (Fig. S1), an area that belongs to a temperate continental climate. The annual average temperature was 8.5°C, while the average precipitation was 450 mm. The soil of the study area is *sierozem* with a weak humus accumulation process, low organic matter content and high calcium accumulation, and a sandy texture. The basic chemical properties of the soil are as pH 7.76, Olsen-P 93.3 mg kg^{-1}, total C 0.25%, total N 0.04%, and electrical conductivity 1015 μs cm^{-1}.

Field experimental design

The potato fermented fertilizer was made of fermenting potato starch wastewater using a strain of *Serratia Marcescens Sakuensis* NR screened from the soil (Zhou et al., 2020) at 15–17℃ for 21 days. The chemical composition of the potato fermented fertilizer was showed in Table S1 and meet the standard of irrigation water quality (GB 5084 – 2021). The field experiment was conducted from 11th February to 7th May 2019. The planted crop was celery, and the variety was *queen*. The area of the field experiment was divided into 21 plots, with each plot covering 8.4 m^2 (2 m wide × 4.2 m long). A randomized block design was used with three replicates. The fertilizer treatments were as follows: 1) CK, no fertilizer; 2) PW1, potato fermented fertilizer 3750 kg•ha^{-1}; 3) PW2, potato fermented fertilizer 7500 kg•ha^{-1}; 4) PW3, potato fermented fertilizer 15000 kg•ha^{-1}; 5) PWF1, potato fermented fertilizer 750 kg•ha^{-1}+ chemical fertilizers; 6) PWF2, potato fermented fertilizer 1500 kg•ha^{-1}+ chemical fertilizers; 7) PWF3, potato fermented fertilizer 3000 kg•ha^{-1}+ chemical fertilizers. The chemical fertilizers (urea, calcium superphosphate, and potassium sulfate) were applied as 450, 150, and 300 kg•ha^{-1} for N, P, and K, respectively before celery was planted. The potato fermented fertilizer were applied ten times through drip irrigation, with the same amount being applied each time. Adjacent plots were separated by a ridge covered with plastic film to prevent seepage and inter-plot movements, and separate irrigation and drainage ditches were provided.

The yield of celery was measured after celery harvest. In addition, five soil core samples from 0–20 cm (topsoil) and 20–40 cm (deep soil) were collected from each plot on 7th May 2019 (after celery harvest). The samples were thoroughly mixed, and the pooled samples were transported on ice to the laboratory within 2–6 h of collection. One sub-samples were air-dried, ground, and sieved through a 2 mm sieve to determine soil properties and soil enzyme activity, while the remaining soil was stored at -80℃ for DNA extraction.

Determination of soil properties and enzyme activity

The soil pH and conductivity were measured using a $CaCl_2$ solution = 1:2.5 (v/v) suspension with a digital pH meter and conductivity meter. The content of exchangeable NH_4^+ was measured using the indophenol blue colorimetric method, while NO_3^- concentration was measured using the KCl extraction for the colorimetric method. Soil available P (Olsen-P) was determined using the 0.5 mol•L^{-1} $NaHCO_3$ extraction, molybdenum antimony anticolorimetric method. On the other hand, soil available K was determined using the NH_4OAC extraction, flame photometry method. The soil was soaked in 0.1mol•L^{-1} HCl, followed by drying and determining total carbon (TC) and total nitrogen (TN) using the elemental analyzer. The soil urease activity was performed using sodium phenate-sodium hypochlorite colorimetry, while soil saccharase and cellulase activities were measured using 3,5-dinitrosalicylic acid colorimetry. Furthermore, the soil catalase activity was measured using potassium permanganate titration described by Cai et al. (2019) and Yu et al. (2019).

Soil DNA extraction, PCR, and MiSeq sequencing

According to the manufacturer's instructions, soil DNA was extracted using OMEGA soil DNA kits. DNA quality was assessed using 0.8% agarose gel electrophoresis, and the DNA was quantified using a Nanodrop 2000 spectrophotometer. Next, the 16S rRNA variable V3-V4 region was amplified using the following primer pair: 515F (5'-GTGYCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). Polymerase chain reaction (PCR) was performed with a final volume of 25 ml containing 5 ml 5X reaction buffer, 5 ml 5X GC buffer, 2 ml dNTP (2.5mM), 1 ml forward primer (10mM), 1 ml reverse primer (10mM), 2 ml DNA template, 8.75 ml ddH_2O, and 0.25 ml Q5 DNA polymerase. The amplification conditions included the following steps: initial denaturation at 98℃ for 2 min; 30 cycles of denaturation at 98℃ for 15 s, annealing at 55℃ for 30 s, and extension at 72℃ for 30 s; and final extension at 72℃ for 5 min, followed by a hold at 10℃. Each sample was amplified several times, and the PCR products were purified after mixing. Finally, the purified PCR product was subjected to a double terminal sequenced analysis using the Illumina MiSeq platform.

Quantitative PCR

The copies of all the functional genes assessed in this experiment were estimated using quantitative real-time PCR (qPCR) in a StepOnePlus real-time PCR system (Applied Biosystems). The PCR mixture contained 10µl of QuantiTect SYBR® Green master mix (Qiagen), 0.5µl of forward and reverse primer each (10µM), and 1µl of DNA template (5 ng μl^{-1}) in a final volume of 20µl. A melt curve analysis was conducted to verify the nonspecific amplification by increasing the temperature from 60 to 95°C with 4.4°C increments every second. Calculation of gene copies in the samples was based on a standard curve generated using standard plasmid DNA, which was made by cloning the target gene fragment as described by Bae et al. (2018). Table S2 shows the primers and conditions for each functional gene.

Statistical analysis

One-way analysis of variance (ANOVA) was used to determine the difference in soil parameters, bacterial community composition, and functional gene copies (Tukey, n = 3), and the results were displayed using Origin 2020. Principal coordinate analysis (PCoA) was used to visualize the dissimilarity of beta diversity based on the Bray-Curtis distance of microbial community profiles. Differentially abundant OTUs were detected using the *DESeq2* package, and the graph was displayed using *ggrepel* and *ggplot2* packages in R. Network analysis was further performed to describe the complex co-occurrence pattern in different treatments, with the relative abundance of OTUs as nodes. Moreover, the results were constructed using *W.G.C.N.A.*, *igraph*, and *RMThreshold* package in R, while the graph was displayed using the Gephi version 0.9.2. Structural equation models are constructed by SPSS Amos. Finally, the original sequence data were deposited in the Genome Sequence Archive with accession number PRJNA727061.

Results

Potato fermented fertilizer affected soil properties and soil enzyme activity

An increase in the application level of potato fermented fertilizer boosts the yield of celery by 165.3-399.4% compared with CK (Table 1). The results showed a significant difference between PWF1, PWF2, PWF3, and PW1, PW2, PW3. This indicates that potato fermented fertilizer plus chemical fertilizer significantly increased celery yield compared to potato fermented fertilizer. Application of potato fermented fertilizer (PW1, PW2, PW3) decreased the total carbon (TC) of top and deep soils by 40.9–50% and total nitrogen (TN) by 40-57.8%, compared with CK. In addition, soil NH_4^+ content in deep soils decreased by 54.3–58% whereas soil NO_3^- content increased by 25.6-109.4%, compared with the levels in the CK (Table 1). Top and deep soils available phosphorus (Olsen-P) and potassium (Av-K) increased, whereas electrical conductivity (EC) decreased gradually with an increase in the level of potato fermented fertilizer. Application of potato fermented fertilizer with chemical fertilizer (PWF1, PWF2, PWF3) did not change the trend of potato fermented fertilizer effects on soil physical and chemical properties. However, the combination fertilizers further increased the content of topsoil Olsen-P by 145.6-166.7%, top and deep soil NO_3^- by 15.2–81.1%, TC by 13.8–14%, and TN by 27.2–34.7% compared with PW1, PW2, PW3 treatments (Table 1).

Table 1
Soil chemical properties after treatments

Depth	Treatment	pH	EC ($\mu s\ cm^{-1}$)	NH_4^+ ($\mu g\ kg^{-1}$)	NO_3^- ($mg\ kg^{-1}$)	Olsen-P ($mg\ kg^{-1}$)	Av-K ($mg\ kg^{-1}$)	FC (%)	TC (%)	TN(%)	yield ($kg\ ha^{-1}$)
Topsoil	CK	7.83 ± 0.02a	1114 ± 104b	52.2 ± 5a	1.76 ± 0.3a	42.3 ± 2.9bc	92.2 ± 6.1c	28.9 ± 1.4b	0.22 ± 0.05a	0.045 ± 0.002a	16020 ± 801c
	PW1	7.76 ± 0.04a	1377 ± 73a	43.6 ± 6.2a	1.4 ± 0.26a	51.8 ± 9.2ab	95.8 ± 3.7c	36.3 ± 2a	0.13 ± 0.08b	0.025 ± 0.003b	42150 ± 2125b
	PW2	7.8 ± 0.01a	1022 ± 29b	42.7 ± 3.1a	2.1 ± 0.65a	38.6 ± 5.2c	114.5 ± 3.1b	36.4 ± 1.4a	0.11 ± 0.08b	0.023 ± 0.003b	43050 ± 2152b
	PW3	7.72 ± 0.03a	780 ± 17c	48.6 ± 4.8a	1.7 ± 0.43a	60.1 ± 1.0a	123.6 ± 1.3a	36.5 ± 1.3a	0.11 ± 0.05b	0.027 ± 0.003b	68406 ± 2420a
	CK	7.83 ± 0.02a	1114 ± 104a	52.2 ± 5a	1.76 ± 0.3c	42.3 ± 2.9b	92.2 ± 6.1a	28.9 ± 1.4c	0.22 ± 0.05a	0.045 ± 0.002a	16020 ± 801c
	PWF1	7.58 ± 0.02b	1037 ± 52ab	40.4 ± 6.3a	2.65 ± 0.23b	147.8 ± 14.1a	99.7 ± 6.5a	40.1 ± 1.4a	0.14 ± 0.03b	0.036 ± 0.003b	66645 ± 3420b
	PWF2	7.58 ± 0.01b	966 ± 16b	59.2 ± 3.1a	3.03 ± 0.39b	151.5 ± 12a	100.4 ± 8.5a	37.8 ± 2.9ab	0.13 ± 0.05b	0.034 ± 0.002b	67710 ± 3380b
	PWF3	7.58 ± 0.01b	1025 ± 10ab	45 ± 4.8a	3.72 ± 0.14a	163.3 ± 18.2a	90.7 ± 2.1a	35.4 ± 1.2bc	0.13 ± 0.04b	0.031 ± 0.002b	80004 ± 3217a
Deep soil	CK	7.7 ± 0.01a	916 ± 2a	81.5 ± 6.5a	1.17 ± 0.04b	29.6 ± 0.8ab	94.4 ± 3.8c	40.1 ± 2.3a	0.24 ± 0.05a	0.042 ± 0.001a	NA
	PW1	7.77 ± 0.02a	813 ± 17b	37.5 ± 7b	2.45 ± 0.36a	32.4 ± 8.8ab	94.7 ± 4.5c	33.9 ± 2.1b	0.14 ± 0.09b	0.026 ± 0.001b	NA
	PW2	7.78 ± 0.2a	753 ± 23c	39.9 ± 2.2b	1.47 ± 0.11b	25.9 ± 2b	107.8 ± 1.6b	36.4 ± 3.1b	0.11 ± 0.05b	0.022 ± 0.002b	NA
	PW3	7.8 ± 0.03a	711 ± 10d	38.4 ± 2.1b	1.94 ± 0.88ab	36.1 ± 2.3a	118.7 ± 3a	37.6 ± 2.2b	0.11 ± 0.0.4b	0.019 ± 0.002c	NA
	CK	7.7 ± 0.01a	916 ± 2a	81.5 ± 6.5a	1.17 ± 0.03c	29.6 ± 0.8b	94.4 ± 3.8a	40 ± 0.2a	0.24 ± 0.05a	0.042 ± 0.001a	NA
	PWF1	7.67 ± 0.01a	711 ± 6c	36.3 ± 4.8b	1.4 ± 0.19c	22.5 ± 2.6c	94.5 ± 0.8a	34.1 ± 1.4c	0.13 ± 0.03b	0.024 ± 0.002b	NA

Depth	Treatment	pH	EC ($\mu s\ cm^{-1}$)	NH_4^+ ($\mu g\ kg^{-1}$)	NO_3^- ($mg\ kg^{-1}$)	Olsen-P ($mg\ kg^{-1}$)	Av-K ($mg\ kg^{-1}$)	FC (%)	TC (%)	TN(%)	yield ($kg\ ha^{-1}$)
	PWF2	7.73 ± 0.03a	785 ± 17b	34.3 ± 5.3b	2.95 ± 0.23a	33.4 ± 4.7b	87.6 ± 2a	37.8 ± 1ab	0.14 ± 0.05b	0.031 ± 0.002b	NA
	PWF3	7.71 ± 0.02a	722 ± 7c	39.1 ± 6.5b	2.39 ± 0.49b	46.3 ± 4.6a	91.7 ± 0.5a	34.8 ± 1bc	0.14 ± 0.05b	0.029 ± 0.002b	NA

Soil urease activity was not significantly affected by PW treatments (Fig. 1). An increase in the level of potato fermented fertilizer gradually increased the activity of saccharase and cellulase by 54.8–71.4% and 21.7–98.7% in PWF1, PWF2, PWF3 treatments compared with CK treatment. In addition, the activity of saccharase and cellulase increased by 11.9–33.3% and 4.8–97.5% after application of PW1, PW2, PW3 treatments, respectively, whereas catalase activity showed the opposite trend at both layers.

Potato fermented fertilizer affected soil bacterial community and composition

The 16S rRNA gene sequencing was performed to explore the effects of different levels and fertilizers treatments on soil microbial community composition. The alpha diversity of the soil bacteria community was characterized by Chao1, ACE, Simpson, and Shannon diversity indices (Table 2). The results showed that potato fermented fertilizer with chemical fertilizer (PWF1, PWF2, PWF3) significantly increased the richness index (Chao1 and ACE) and decreased the diversity index (Simpson and Shannon) of topsoil and deep soil compared with administration of CK. Moreover, principal coordinate analysis (PCoA) showed the contribution rate of the first row of PW1, PW2, PW3, CK and PWF1, PWF2, PWF3, CK treatments of deep soil (56.74% and 54.3%) were significantly higher compared with that of topsoil (27.6% and 34.61%) (Fig. 2). Application of potato fermented fertilizer was significantly correlated with the beta diversity of the soil bacteria community of top and deep soil in PW1, PW2, PW3, CK treatments ($R = 0.76$, $P < 0.01$; $R = 0.66$, $P < 0.01$) and PWF1, PWF2, PWF3, CK treatments ($R = 0.96$, $P < 0.01$; $R = 0.98$, $P < 0.01$). Moreover, applications of different levels of potato fermented fertilizer ($R = 0.93$, $P < 0.01$ in PW1, PW2, PW3 treatments; $R = 0.95$, $P < 0.01$ in PWF1, PWF2, PWF3 treatments) showed significant differences in beta diversity of the deep soil bacteria community. However, the analysis did not show a significant difference between PW1, PW2, PW3 treatments ($R = 0.49$, $P = 0.011$), while a significant difference was observed in topsoil under PWF1, PWF2, and PWF3 treatments ($R = 0.77$, $P < 0.01$).

Table 2
Effects of potato fermented fertilizer on soil microbial community diversity

Depth	Treatment	Chao1	ACE	Simpson	Shannon
Topsoil	CK	4437.9 ± 113.1c	6173.3 ± 129.2b	0.01 ± 0.001c	2.83 ± 0.02ab
	PW1	5006.7 ± 316.3a	6798.1 ± 332.7a	0.0126 ± 0.001b	2.86 ± 0.02a
	PW2	4818.7 ± 50.4ba	6694.9 ± 77.6a	0.015 ± 0.002a	2.83 ± 0.01b
	PW3	4554.9 ± 145.7bc	6305.2 ± 34.3b	0.009 ± 0.001c	2.86 ± 0.01a
	CK	4437.9 ± 113.1a	6173.2 ± 129.2ab	0.01 ± 0.001b	2.83 ± 0.02a
	PWF1	4265.6 ± 61.3a	6045.3 ± 70.7b	0.014 ± 0.002a	2.78 ± 0.02b
	PWF2	4667 ± 454.7a	6546.8 ± 417a	0.015 ± 0.002a	2.83 ± 0.02a
	PWF3	4356.3 ± 152.7a	6110.2 ± 142.5ab	0.013 ± 0.001a	2.79 ± 0.01b
Deep soil	CK	4362.4 ± 207.6b	6075.6 ± 270.2b	0.01 ± 0.001b	2.8 ± 0.02b
	PW1	4876.6 ± 281.4a	6786.1 ± 299.2a	0.02 ± 0.004a	2.78 ± 0.04b
	PW2	4940.8 ± 61.4a	6839.4 ± 118.5a	0.02 ± 0.003a	2.8 ± 0.04b
	PW3	5106.3 ± 82.8a	6924.9 ± 129.5a	0.01 ± 0.001b	2.87 ± 0.01a
	CK	4362.4 ± 207.6c	6075.6 ± 270.2b	0.01 ± 0.001b	2.8 ± 0.02b
	PWF1	4684.7 ± 44.8b	6581.9 ± 63.9a	0.018 ± 0.001a	2.8 ± 0.01b
	PWF2	4309.8 ± 128.7c	6102.9 ± 251b	0.017 ± 0.002a	2.78 ± 0.02c
	PWF3	5035.7 ± 30.6a	6897 ± 100a	0.018 ± 0.001a	2.84 ± 0.01a

Operational taxonomic units (OTUs) are showed in Fig. 3 which generated based on similarity of mainly clustered PCR amplified 16S sequences. Application of potato fertilizer treatments significantly upgraded or downgraded OTUs, including amounts and kinds of different OTUs compared with CK (Fig. 3). However, the application of potato fermented fertilizer significantly increased the abundance of *Arthrobacter* (OTU1), *Pseudomonas* (OTU83), and *Azoarcus* (OTU32), which are involved in carbon mineralization and N fixation (Karigar et al., 2006; Chevalier et al., 2017; Sperfeld et al., 2018). On the contrary, application of potato fermented fertilizer significantly decreased the abundance of *Nitrosospira* (OTU34), *Nitrolancea* (OTU518), and *Aquicella* (OTU1227) (Fig S2), which are nitrifying and pathogenic bacteria (Santos et al., 2003; Norton et al., 2008; Spieck et al., 2020). Effects on different OTUs in all treatments at topsoils were similar (Fig. 3a-f). The number, type, and degree of difference OTUs increased gradually with increased levels of potato fermented fertilizer at deep soils (Fig. 3g-i). However, the number, type, and degree of difference of OTUs in PWF1, PWF2, PWF3 treatments were similar to PW3 treatment, which was the highest compared with PW1 and PW2 treatments (Fig. 3j-l). Covariance network analysis shows that applying potato fermented fertilizer decreases the number of nodes, edges, modularity and average clustering coefficient of the network, and increases the weighted average degree and the proportion of positively correlated edges (Fig. 4). The addition of fertilizer continued to exacerbate these changes in the network. This suggests that the addition of potato fermented fertilizer reduces the diversity of soil microorganisms, causing the microbial community to evolve in a direction that is more suitable for the environment in which potato fermented fertilizer is added, and reduces the competitive relationships in the community making it more stable. These results indicate that the application of potato fermented fertilizer significantly affected soil microorganisms and microbial communities in deep soil whatever applied with or without chemical fertilizer. Moreover, chemical fertilizer can improve the effect of potato fermented fertilizer on the soil microbial community.

Potato fermented fertilizer modulates soil nitrification process

The above results showed that NO_3^- the content of topsoil was significantly increased after increased application PWF levels but not in PW levels (Table 1), indicates that chemical fertilizer can affect the conversion of organic N to inorganic N and the nitrification process. Therefore, the abundance of functional genes of essential microorganisms implicated in the nitrification process, including ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), comammox *Nitrospira* clade A and B, and nitrite-oxidizing bacteria (NOB) were explored (Fig. 5). The results showed that the abundance of AOB and NOB decreased with the increase level of potato fermented fertilizer. It is interesting that a significant increase in abundance of AOA and comammox (COM) was observed in PW1, PW2, PW3, and PWF1, PWF2, PWF3 treatments at deep soils, but the effect was not significant for the application of PW1, PW2, PW3 treatments on topsoil. The abundance of AOA significantly decreased, and COM significantly increased at the topsoil after applying PWF1, PWF2, PWF3 treatments. These results suggested that potato fermented fertilizer significantly affected the microbial community in deep soil in this study.

Correlations between N cycling functional genes, environmental factors, and input of potato fermented fertilizer (Input) in deep soils were further explored (Fig. 6). The results showed a significant positive correlation between copies of AOA and COM ($r = 0.79$, $P < 0.01$), AOB, and NOB ($r = 0.7$, $P < 0.05$) in PW1, PW2, PW3 treatments (Fig. 6a). Furthermore, a significant positive correlation was observed between copies of AOA, COM, and Input ($r = 0.72$, $P < 0.01$; $r = 0.67$, $P < 0.05$) but not in AOB, NOB, and Input. In PWF1, PWF2, PWF3 treatments (Fig. 6b), a significant positive correlation was observed between NOB and AOB ($r = 0.64$, $P < 0.05$), Input and COM ($r = 0.69$, $P < 0.05$), while no correlation was observed between AOA and COM, AOA and Input. A significant negative correlation was observed between AOA and AOB ($R=-0.81$, $P < 0.01$). Based on the correlation between potato fermented fertilizer application and AOA and comammox, we further analysed their relationship with yield by means of structural equation modelling, which indicated that potato fermented fertilizer application increased the nitrate-nitrogen content of the soil by increasing the abundance of comammox, while potato fermented fertilizer application also increased the microbial load of the soil, which increases led to an increase in celery yield (Fig. 7).

Discussion

The fermented potato fertilizer changed soil properties and enzyme activity

Potato wastewater contains significant amounts of mineral compounds (approximately 1%), which are dominated by potassium (K) and phosphorus (P), and proteins, including patatin, alkaline inhibitors of proteases, and complex 22kDa proteins (Markiewicz et al., 2015). Kurcz et al. (2016) reported that glutamic and aspartic acid's highest share of amino acids is taken up. Therefore, the application of potato fermented fertilizer significantly increases soil Olsen-P and Av-K at the topsoil and deep soil. Notably, P is easily fixed in alkaline soil, while K is easily lost in alkaline soil, which can be attributed to the fact that the fluidity of P and K in soil is very different. Thus, most of the P in PWF1, PWF2, and PWF3 treatments were fixed on the surface, while most of the K was lost during irrigation, which explains why the soil Av-K was lower than PW1, PW2, and PW3 treatments.

The study site was Yinchuan, Ningxia, China, an area with *sierozem* soil with a weak humus accumulation, low organic matter content, and calcium accumulation, indicating that the soil is poor. Results showed that the soil TC content decreased when potato fermented fertilizer was applied without chemical fertilizer (PW1, PW2, and PW3 treatments) but increased significantly in PWF1, PWF2, and PWF3 treatments compared to the PW1, PW2, and PW3 treatments. Fontaine et al. (2010) reported that the supply of fresh carbon might accelerate soil carbon decomposition and induce a

negative carbon balance because of the lack of energy available to soil microorganisms. Moreover, Li et al. (2019a) reported that short-term N-addition could significantly increase the soil respiration and its components, C and N, storage in soil. Therefore, potato fermented fertilizer with chemical fertilizer increased the content of nutrients that are rapidly utilized by microorganisms, thereby accelerating the utilization of potato fermented fertilizer by soil microorganisms and increasing the storage of soil carbon and nitrogen.

Previous studies have indicated that soil urease, saccharase, cellulase, and catalase are associated with the soil health, carbon cycle, and antioxidant defense system (Zhang et al., 2014; Kaya et al., 2019; Liu et al., 2020a). In this study, there was no significant change of urease in each treatment, suggesting that potato fermented fertilizer has no adverse effects on soil biochemical functions. Liang et al. (2018) reported that potato fermented fertilizer contains a large amount of soluble organic carbon, stimulating the soil carbon cycle (activity of saccharase and cellulase) when applied to the soil. At the same time, the decomposition ability of soil to hydrogen peroxide (the activity of catalase) was decreased after the input of organic carbon (Fig. 1), which is consistent with the results reported by Bissey et al. (2006). The increase in the activity of soil saccharase and cellulase in PWF1, PWF2, and PWF3 treatments was more significant than that in PW1, PW2, and PW3 treatments, indicating that potato fermented fertilizer with chemical fertilizer has a more vital ability to decompose organic matter and sequester carbon than potato fermented fertilizer alone. Soil available N is the crucial factor affecting soil carbon cycle and priming effect (Li et al., 2018), and the addition of P stimulates soil organic matter priming by inducing microbial demand for N and stimulating the growth of soil organic matter degrading populations (Mehnaz et al., 2019). Therefore, it can be concluded that the available nutrients in the chemical fertilizer can induce soil microorganisms to utilize potato fermented fertilizer fully.

The fermented potato fertilizer changed the soil microbial community structure in deep soils

Results showed that potato fermented fertilizer significantly increased the richness of the soil microbial community (Table 2). However, the diversity of the soil microbial community was decreased, which might have been caused by the decrease of soil catalase activity. This can be attributed to the fact that decreased catalase activity results in the accumulation of H_2O_2 in soil, which is highly toxic to many groups of microorganisms in the soil (Kakosová et al., 2017), ultimately leading to the decrease of soil microbial diversity. The results of soil microbial richness index, PCoA, differential OTUs, and topological properties of network analysis suggested that application of potato fermented fertilizer could significantly change the structure of soil microbial community, and the changes were more significant in deep soils. This can be explained by the potato fermented fertilizer infiltrating faster to deep soils when it was applied to the surface soil due to the sandy soil of research area. Thus, the potato fermented fertilizer had a more significant effect on deep soils. Moreover, the results indicated that the potato fermented fertilizer, especially with chemical fertilizer, can significantly differ between different levels (Fig. 2) and stimulate differentiation of OTUs (Fig. 3). It can also significantly increase the network edge, node, network fragmentation and stability (Fig. 4). These results further confirmed the above conclusion that available nutrients in chemical fertilizers can induce soil microorganisms to utilize potato fermented fertilizer fully.

Several previous studies have reported that application of organic and inorganic fertilizers can influence the soil biochemical attributes, soil microbial community composition and abundance, increase soil bacterial and fungi community, to build a stable soil environment, and inhibit the occurrence of plant diseases compared to the application of organic or inorganic fertilizers alone (Leite et al., 2017; Ali et al., 2019; Li et al., 2019b; Tang et al., 2020). Most soil microorganisms can directly utilize the available N and P from fertilizers, including autotrophic, heterotrophic, and mixotrophic microorganisms that can use organic carbon and CO_2 as carbon sources. However, microorganisms require much more C than N and P. Therefore, when there are many available N and P in the soil system, soil microorganisms will be stimulated to decompose a large amount of organic carbon or fix CO_2 in the air. This can explain why short-term

N fertilizers can significantly increase the soil respiration C storage in soil and shift from a more oligotrophic bacterial community to one that is more eutrophic (Fierer et al., 2012; Li et al., 2019a).

The fermented potato fertilizer changed soil nitrification strategy by changing the niche of soil functional microorganisms

Results indicated that the application of potato fermented fertilizer, especially with chemical fertilizer, can promote soil nitrification. The application of potato fermented fertilizer alone treatments shaped soil organic nutrient environment, while the application of potato fermented fertilizer with chemical fertilizer treatments shaped mixed (organic plus inorganic) nutrient environments. Therefore, we hypothesize that there are different strategies for soil nitrification in the above two nutrient environments. The results obtained in this study have shown that the abundance of AOA and COM increased in these two environments while the abundance of AOB decreased. However, AOA and COM had positive correlations in the organic nutrient environment, and no correlation was observed in the mixed nutrient environment. The order of ammonium affinity for these three functional genes was COM > AOA > AOB, which suggests that COM is more suitable for surviving in oligotrophic soils (Kits et al., 2017; Sakoula et al., 2020). It is worth noting that organic nutrient environments have a relatively low ammonium concentration because the ammonium is derived from the mineralization of organic matter, not directly from chemical fertilizer. Daims *et al.* (2015b) reported that both AOA and COM use ammonium as the substrate in N transformation. Therefore, in theory, there is a competitive relationship between AOA and COM; however, this study's results showed a significant positive correlation ($R = 0.79$, $P < 0.01$) between the copies of AOA and COM. Thus, we proposed two hypotheses based on this result: (1) AOA directly utilizes the organic matter in potato fermented fertilizer. Multiple studies have shown that some AOA may have mixed or heterotrophic growth strategies (Prosser and Nicol, 2008; Jia and Conrad, 2009; Liu et al., 2018). In the organic nutrient environment, AOA participates in the decomposition of organic matter and COM using ammonium obtained from the mineralization of organic matter. This explains why there was a positive correlation between them and their abundance increased with the addition of organic matter; (2) COM makes use of nitrite nitrogen produced by AOA nitrification. Koch et al. (2019) reported that COM is genetically capable of using nitrite nitrogen, which explains why AOA and COM were positively correlated.

The addition of urea in a mixed nutrient environment increases the content of available soil N, thereby AOA and AOB compete for the ammonium produced by urea hydrolysis. Xue et al. (2016) reported that AOA has more competitive advantages under mixed nutrition conditions compared to AOB, which is consistent with our results of a negative correlation between the abundance of AOA and AOB ($R=-0.81$, $P < 0.01$). Furthermore, COM still uses the ammonium obtained from the mineralization of organic matter in areas with a low ammonium concentration in the soil.

Conclusions

This study showed potato fermented fertilizer stimulate the growth and differentiation of soil microorganisms. The two fertilization formulas (potato fermented fertilizer without or with chemical fertilizer) shaped the different soil nutrient environments, thereby changing the niche of AOA, AOB, and comammox (including clade A and B) in the study soil. Soil functional microorganisms AOA and comammox may have a mutually beneficial relationship in an organic nutrient environment shaped by potato fermented fertilizer, which is replaced by a competitive relationship between AOA and AOB in a mixed nutrient environment shaped by potato fertilizer with chemical fertilizer. The results indicated that potato fermented fertilizer provides the necessary nutrients for plant growth, either directly or by stimulating the nitrification process in the soil, increasing yields.

Declarations

Acknowledgments

The authors gratefully acknowledge the financial support from the National Key Research and Development Program of China (2021YFD1700803), the Natural Science Foundation of Zhejiang Province (LZ21C030002) and the National Natural Science Foundation of China (41877044).

Compliance with ethical standards

Conflict of interest: The authors declare no conflict of interest.

Ethical approval: This article does not contain any experiments involving human participants or animals performed by any authors; thus, ethical approval is not necessary

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Figures

Figure 1

Soil enzyme activities under different depth and application amount of potato wastewater. (a) Soil enzyme activities of PW1, PW2, PW3 treatments at topsoil. (b) Soil enzyme activities of PW1, PW2, PW3 treatments at deep soil. (c) Soil enzyme activities of PWF1, PWF2, PWF3 treatments at topsoil. (d) Soil enzyme activities of PWF1, PWF2, PWF3 treatments at deep soil.

Note: Different lowercase letters indicate significant difference in activity of the same enzyme among the different treatments ($n = 3$, $P < 0.05$).

Figure 2

Principal coordinate analysis of β diversity of every treatment. (a) PW1, PW2, PW3 treatments at topsoil. (b) PW1, PW2, PW3 treatments at deep soil. (c) PWF1, PWF2, PWF3 treatments at topsoil. (d) PWF1, PWF2, PWF3 treatments at deep soil.

Figure 3

Box line diagram and volcano map of differentially expressed OTU between CK and PW1(a), PW2(b), PW3(c), between CK and PWF1 (d), PWF2(e), PWF3(f) at topsoils. Differentially expressed OTU between CK and PW1(g), PW2(h), PW3(i), between CK and PWF1 (j), PWF2(k), PWF3(l) at deep soils.

Figure 4

Analysis of covariance networks of treatments CK, PW, PWF. (a) networks of treatments CK, PW, PWF. (b) Relative species abundance at phylum in network. (c) Topological properties of covariance networks of treatment CK, PW, PWF.

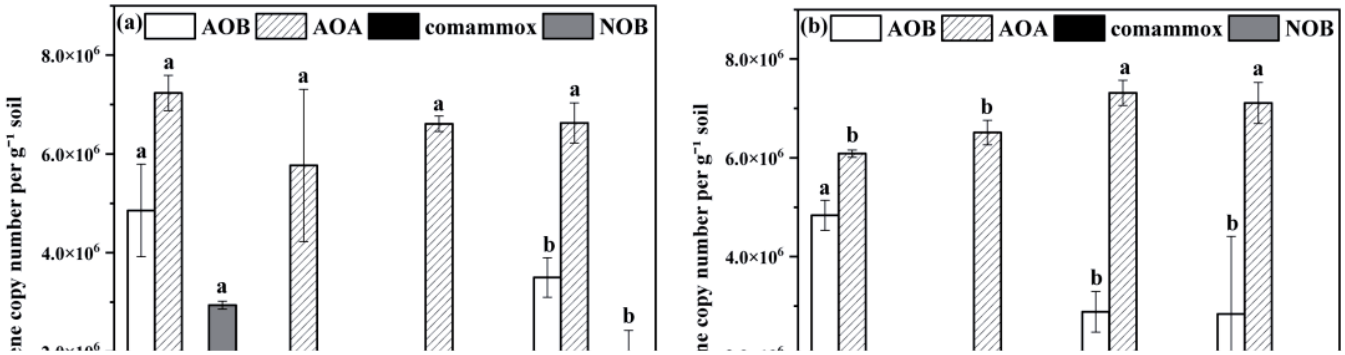

Figure 5

Copies of AOA, AOB, NOB and comammox under different fertilizer treatments. (a) PW1, PW2, PW3 treatments at topsoil. (b) PW1, PW2, PW3 treatments at deep soil. (c) PWF1, PWF2, PWF3 treatments at topsoil. (d) PWF1, PWF2, PWF3 treatments at deep soil.

Note: Different lowercase letters indicate a significant difference in the same gene among the different treatments (n = 3, $P < 0.05$).

Figure 6

The heat maps of correlations between environmental factors and nitrogen cycling functional genes. (a) PW1, PW2, PW3 treatments at deep soil. (b) PWF1, PWF2, PWF3 treatments at deep soil.

Note: *** indicate a significant difference $P < 0.01$; ** indicate a significant difference $P < 0.05$.

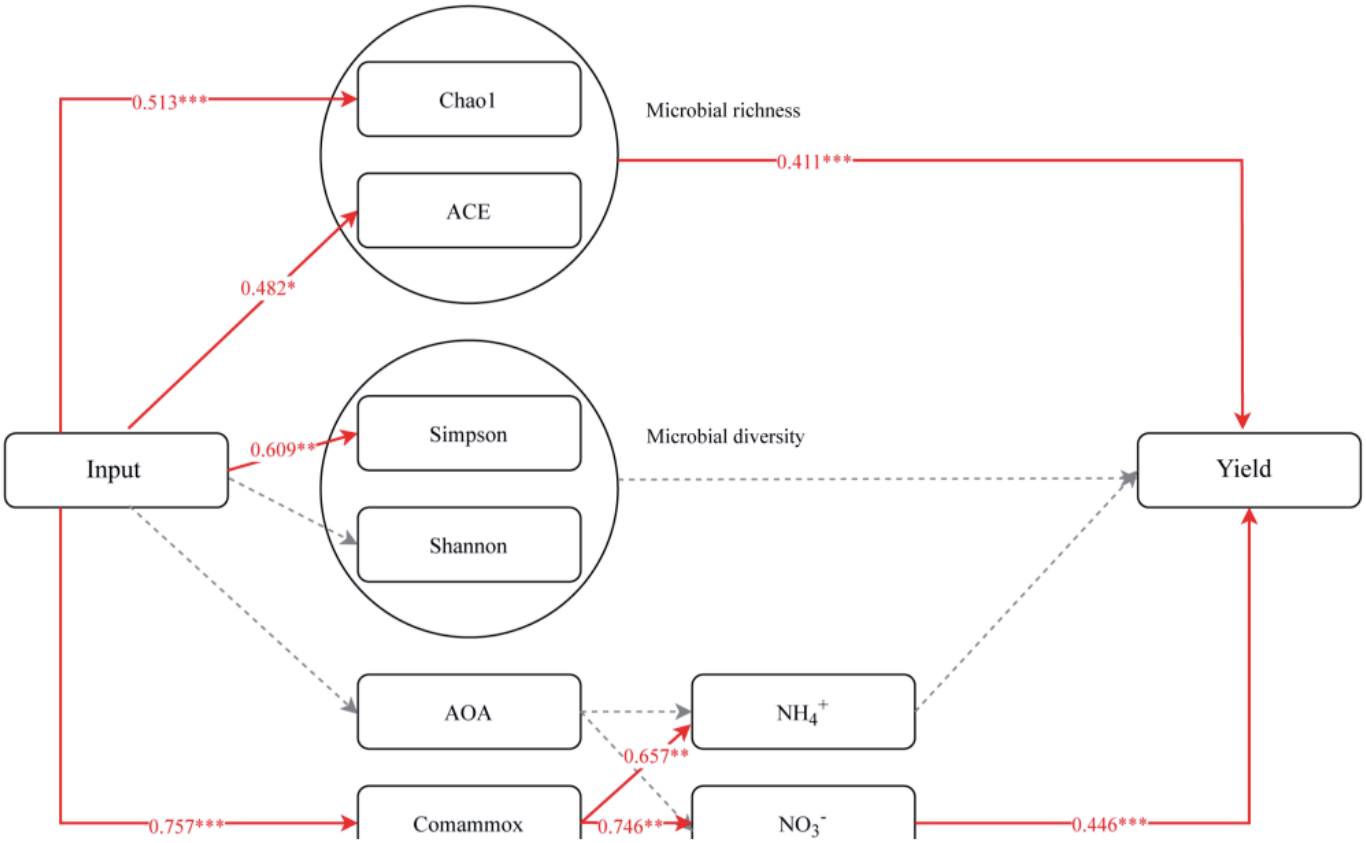

Figure 7

Structural equation modelling between DPW application rate and yield.

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