Brief Report

Effects of Microplastics Addition on Soil Available Nitrogen in Farmland Soil

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Abstract: As microplastics (MPs) are an emerging pollution to farmland ecosystems, the research into the ecological and environmental effects of MPs need to be clarified urgently. Available nitrogen is the determining factor for productivity in most terrestrial ecosystems, especially for the farmland ecosystems with a high productivity. To explore the effects of MPs on soil available nitrogen in farmland soil, an incubation experiment was conducted by adding polyethylene MPs with different concentrations to farmland soil, which was collected from farmland in Fuqing, Fujian Province. The contents of three different nitrogen forms (nitrate, ammonium, and dissolved organic nitrogen) and soil dissolved organic carbon were measured, and the soil mineralization rate was calculated. Bacteria was quantified and bacterial community diversity indexes were measured. The results showed that the MPs addition (T1 and T2) had no significant effect on soil ammonium, nitrate and soil nitrogen mineralization rate compared to the control ($p > 0.05$). However, a significant increase was observed in soil dissolved organic carbon and dissolved organic nitrogen content ($p < 0.05$). It can be seen that the influence of PE MPs on the soil dissolved organic nitrogen is greater than that of inorganic nitrogen. The results of this study showed no major detrimental effects of MPs on the abundance of some bacterial families, whereas a significant change in soil bacterial evenness index was observed in T2 treatment compared to the treatment without MPs addition. In the background of current MPs pollution, the research results can provide a scientific basis for reducing nitrogen loss in soil and protecting farmland soil safety.

Keywords: microplastics; farmland soil; available nitrogen; nitrogen mineralization rate; microbial diversity

1. Introduction

Microplastics (MPs) represent an emerging pollutant to the global ecological environment [1]. Its high surface curvature could contribute to a high loading capacity for persistent organic pollutants and heavy metal [2,3]. The ecological effects of MPs have attracted considerable attention in recent years [4–7] and the deleterious effects on the ocean has received overwhelming attention compared to the terrestrial ecosystem [8,9]. Nevertheless, most plastic debris were released into terrestrial ecosystems, as has been reported. The amount of MPs on terrestrial environments was 4–23 folds larger than in the maritime ecosystem [10]. Indeed, Nizzetto et al. [11] suggested that farmland soils alone might accumulate more MPs than ocean. MPs presence is ubiquitous in terrestrial ecosystems as has several potential impacts for the ecosystem structure and function as well as for human health. For example, physiochemical alternations triggered by MPs could change soil microbe diversity, potentially influencing N fixing association in soil ecosystem [1]. Additionally, MPs exposure in humans could result in inflammatory and

gene damage [12]. Therefore, it is urgently needed to investigate the deleterious ecological impacts of such small plastic particles in agricultural soil.

Nitrogen (N) is considered to be the primary growth-limited factor in various terrestrial ecosystems [13], especially for the agricultural ecosystem with a high productivity. The fact is that N in the soil mainly exists in the form of organic N, which is difficult to be absorbed by plants. Thus, the soil N mineralization rate plays a key role in N availability because plants can utilize the organic N when it is mineralized into inorganic N [14]. MPs arriving in agricultural systems can accumulated in the soil, resulting in changes in soil physical and biological properties, such as soil porosity, air circulation and carbon (C) [7,15]. These alternations in the soil structure and function driven by MPs could affect soil microbial activity and biodiversity [16,17], with potential impacts on soil N availability and mineralization [18]. At present, little research about the effects of MPs on soil available N has been conducted, but these studies yielded some inconsistent results. For example, Liu et al. [19] observed a significant change in soil soluble organic N (DON), soluble organic carbon (DOC) and nitrate N (NO_3^--N) after the addition of 28% (w/w) polypropylene (PP) MPs (<180 μm) to a losses soil compared to the control. Chen et al. [20] suggested that the small pieces of a biological plastic termed polyactic acid (PLA) could decrease the concentration of ammonium, while increasing the contents of nitrate in soil. Boots et al. [21] indicated that the addition of polyethylene (PE) MPs affected the microbial activity related to N cycling and reducing N availability. The study by Green et al. [22] indicated that the present of PE microplastics can inhibit the activities of key enzymes in the soil N cycle, and then affect the soil N cycle. It has also been reported that PP MPs can directly inhibit N availability by chelating ammonium N in the soil through their surface functional groups [23]. The reason for these differences in results may be attributed to MPs type, morphology and their dose. To the best of our knowledge, the potential changes in soil biophysical properties triggered by MPs contamination have not been studied.

Plastics films are widely used in agriculture practice to maintain and conserve soil temperature and moisture around the world [24]. The global market for agricultural films was estimated at 4.6 million tons in 2013, and was expected to grow at a rate of 6.0% per year over the period 2013–2020 [25]. China is the largest consumer and producer of agricultural films. The usage of plastic films increased dramatically in China from 0.32 to 1.25 million tonnes from 1991 to 2011 [26]. PE is the most dominating raw material for agricultural films segment and accounted for about 60% of total market volume in 2013, potentially a major resource of MPs in farmland soil [25,27]. About 1270–2130 tons of MPs particles per million habitants per year were reported to be generated in European urban areas, while an annually MPs addition from sludge in European agricultural sites ups to 63,000–43,000 tons [5]. Around 7100–42,900 MPs particles per kg was estimated to be accumulated in agricultural soil in China [28]. The majority of MPs particle size is between 50 μm and 1 mm, accounting for 95% of the total MPs arriving in the agricultural soil [28].

To investigate the effects of MPs on soil available N in farmland soil, an incubation experiment was conducted by adding PE MPs (125–150μm) with different concentrations to farmland soil collected from a farmland in Fuqing, Fujian Province. This study analyzed the contents of three different N forms (nitrate, ammonium, and dissolved organic N (DON)) and soil dissolved organic carbon (DOC), and calculated the soil mineralization rate. The microbial diversity was also measured to illustrate how MPs alter the soil microbial community and functions [29]. The present study aims at revealing the influence of MPs on N availability in farmland soil and provides a scientific basis for risk management and control of farmland soil from MPs pollution.

2. Materials and Methods

2.1. Soil Sampling

The soil used in this experiment were sampled in Jiangjing Modern Agriculture Garden (25.5° N and 119.4° E) in Fujian Province, China, at an elevation of 4.6 m above sea level. This area climate is characterized by a subtropical monsoon climate with a mean annual

temperature of 21.1 °C and an annual rainfall of 1450 mm. This soil type is sandy loam. The soil samples were collected in November 2021 after the pepper being harvested. Five sample soil cores (upper 0–15 cm) were randomly collected in a 20 m × 20 m plot in the above-mentioned agricultural garden. The collected soil was stored in a deepfreeze and transported to laboratory at the same day. After removing stones and plant residues, soil samples sieved on 2 mm to remove fine roots and divided into two subsamples: One part was used for the analysis of soil physical and chemical properties, and the other was stored in a refrigerator for the following incubation experiments. The soil properties are shown in Table 1.

Table 1. Soil basic properties.

pH	BD (g·cm^{-3})	SWC (%)	TOC (%)	TN (%)	NH$_4^+$ (mgN·kg^{-1})	NO$_3^-$ (mgN·kg^{-1})
6.58 ± 0.17	1.33 ± 0.08	18.3 ± 2.1	1.36 ± 0.15	0.22 ± 0.02	8.71 ± 0.41	8.71 ± 0.53

Note: BD means bulk density; SWC means Soil Water content; TOC means total organic carbon; TN means: tota nitrogen.

2.2. Experimental Design

Three treatments were established in this study: (1) CK: control (no MPs added); (2) T1: 1 g of MPs added (1% *w/w*); and (3) T2, 5 g MPs added (5% *w/w*). The MPs used in this experiment is made of Polyethylene (Cospheric, CA, USA). The MPs concentrations added into soil was based on the previous studies where MPs were observed to significantly accumulate up to 7% in the topsoil [30,31]. The soil and PE MPs were evenly mixed and placed in sterilized glass pots. The density of MPs is 0.96 g/cm^3 and the particle size is 125 μm–150 μm. MPs were sterilized 3–4 times by using 95% absolute alcohol to remove other organic matter on the surface of MPs particles before adding to soil. The soils were slightly compacted by free gravity falling. The soil moisture was kept at 60% of field capacity throughout the experiment. The pots were covered by cling-film with some tiny holes to keep gas exchange, and then were incubated in a dark environment at 25 °C and a relative humidity of 75%. Samples from each pot were removed on 1, 2, 4, 6, 10, 15 and 30 days. Each treatment had five replicates. The soil samples were separated to three groups. One was kept in a refrigerator awaiting analysis for soil NH$_4^+$, NO$_3^-$ and soil organic N. Another group was used to test soil basic properties. The third group was stored at −20 °C for the analysis of soil microbe.

2.3. Soil Physicochemical Properties

Soil pH was determined by using a pH meter (PHS-3C pH meter, Rex, Shanghai, China) in a 1:2.5 soil to water ratio. Total organic carbon (TOC) and total N (TN) were measure by using an element analyzer (Vario Isotope Cube, Germany). Mineral N was extracted with 2 M KCl at a soil-to-solution ratio of 1:5. The concentration of NO$_3^-$ and NH$_4^+$ were measured by a discrete chemistry analyzer (SmartChem 200, AMS Alliance, Rome, Italy). Soil DON and DOC were extracted with 0.5 mol L^{-1}K$_2$SO$_4$ and quantified using a TOC analyzer (Shimadzu, Kyoto, Japan). Net N mineralization rate was measured as the difference between the final and initial inorganic N contents divided by the number of incubation days.

2.4. Soil Microbial Diversity

Soil samples were extracted to quantify the bacterial community diversity using high-throughput sequencing technology. The 0.5 g soil was extracted by a soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA). The hypervariable region V4-V5 of bacterial 16S rRNA genes were amplified with primer 515F (GTGCCAGCMGCCGCGG) and 907R (CCGT-CAATTCMTTTRAGTTT). The conditions and procedures of Polymerase Chain Reaction (PCR) amplification are referred to this paper from Fei et al. [32], and the PCR quantification was performed, followed by the illustration from Wang et al. [33]. Purified amplicons were

sequenced with an Illumina MiSeq platform (Illumina, San Diego, CA, USA) according to the standard protocols provided by Majorbio BioPharm Technology Co. Ltd. (Shanghai, China). The raw bacterial sequence in this study were deposited in National Center for Biotechnology Information (NCBI) Sequence Read Archive database (SUB12195744).

Alpha community diversity analysis (i.e., Chao1/ASV richness indexes, evenness indices and Shannon diversity estimator) was analyzed by applying the operational taxonomic unit (OTU) table in Quantitative Insights Into Microbial Ecology toolkit (QIIME). The indexes of diversity (Chao1, ASV, Shannon and Evenness indices) were analyzed.

2.5. Statistical Analysis

Values shown in figures and tables were presented with mean value (±standard deviation, SD). One-way ANOVA was used to determine the differences in soil DIN, DON, DOC and mineralization rates among treatments with a least significant difference (LSD) multiple comparisons test. Pearson correlation analysis was employed to test degrees of correlation among soil N mineralization rate and NH_4^+, NO_3^-, DON, DOC. All statistical analyses and figures were performed using Origin 8.5 (OriginLab Corporation, Northampton, MA, USA).

3. Results

3.1. The Effects of MPs on Soil Inorganic N and Mineralization Rate

There was no significant difference in NH_4^+ content among CK, T1 and T2 during the incubation period ($p > 0.05$) (Figure 1A). Similar to NH_4^+, NO_3^- contents except day 1, were observed to have no significant change after MPs addition (Figure 1B). The NO_3^- content was higher in T1 (13.58 ± 1.27 mgN·kg^{-1}) and significantly lower in T2 than CK at the first day after MPs addition. Then nitrate contents declined quickly to 9.18 ± 0.09 mg N·kg^{-1} between days one and two among three treatments, following by a fluctuation from day two until the end of incubation. MPs additions had no significant effects on the soil net N mineralization rate in comparison to CK ($p > 0.05$).
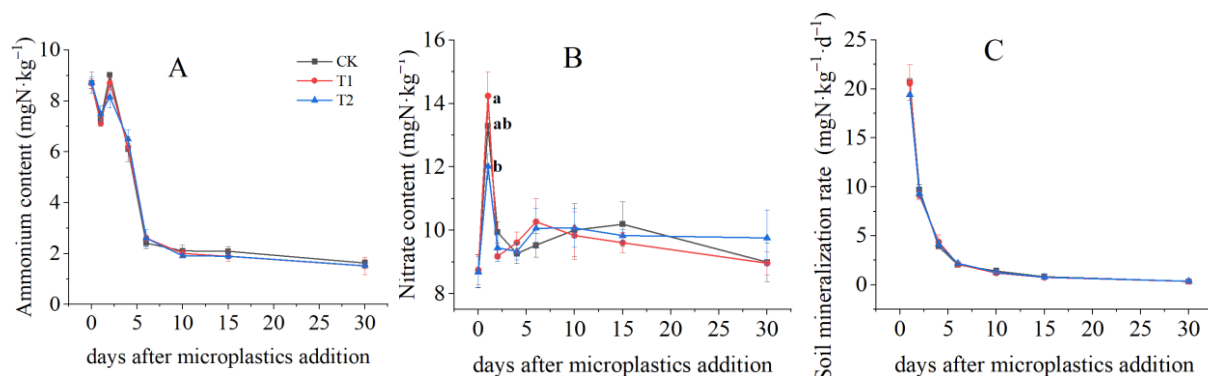

Figure 1. Temporal variations of soil NH_4^+ (**A**), NO_3^- (**B**) contents and net mineralization rate (**C**). Letters designate significant differences among treatments ($p < 0.05$).

3.2. The Effects of MPs on Soil DON and DOC

MPs addition significantly affected DON contents ($p < 0.05$) (Figure 2A). MPs enhanced the release of DON throughout the incubation, as compared to the CK treatment. A significant increase ($p < 0.05$) of DON content was observed in T2 than T1 during the first six days after MPs addition. At day 6, DON content in T2 and T1 increased to 30.5 mg N·kg^{-1} and 33.7 mg N·kg^{-1}, respectively, relative to CK. Between days 6 and 15, DON contents in T1 and T2 showed a significant decline tendency. The DON content decreased by 3.3% in T1 and 32.9% in T2. While increases of DON were observed in all the treatments from day 15. On day 30, the DON content was the highest in T1 (38.7 mg N·kg^{-1}), followed by T2 (33.0 mg N·kg^{-1}).
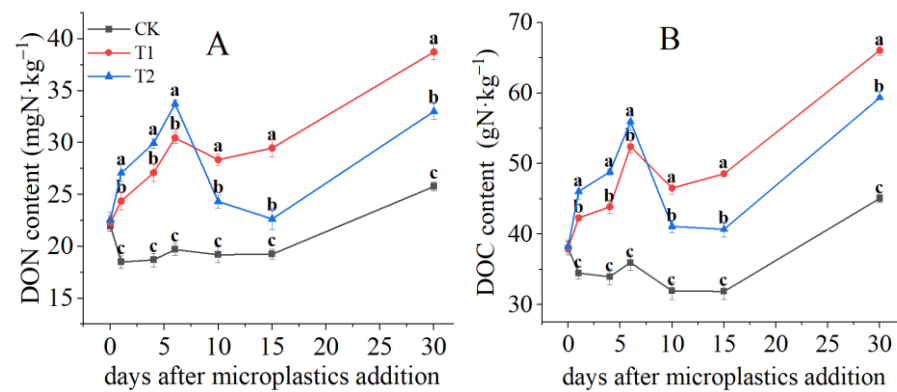
Figure 2. Temporal variations of soil DON (**A**) and DOC contents (**B**). Letters designate significant differences among treatments ($p < 0.05$).

Soil DOC content showed a similar tendency with DON (Figure 2B). Compared to CK, the DOC content increased significantly in T2 and T1. Dramatic decline was observed at T2 and T1 from day 6 to day 15. The DOC content decreased to 40.7 g N·kg^{-1} and 48.6 g N·kg^{-1} in T2 and T1, respectively. Then, DOC content increased from day 15 to day 30 in all treatments. On day 30, the highest amount of DOC content was in T1 (66.13 g N·kg^{-1}), followed by T2 (59.4 g N·kg^{-1}).

3.3. Bacterial Community Diversity

As shown in Table 2, ASV, chao1 and Shannon indices did not show a significant difference ($p > 0.05$) in these three different treatments. While a significant difference was observed between the soil absent of MPs and 5% PE (T2) in Evenness index ($p < 0.05$). Nonetheless, there is no significant difference in the evenness index values between CK and T1 treatments.

Table 2. Effects of MPs on the diversity of bacteria community.

Treatment	ASV Richness	Shannon	Chao1	Evenness
CK	2085.36 ± 109.02 [a]	9.73 ± 0.11 [a]	2324.14 ± 195.85 [a]	0.144 ± 0.014 [a]
T1	2051.80 ± 122.09 [a]	9.70 ± 0.10 [a]	2261.32 ± 197.04 [a]	0.143 ± 0.012 [ab]
T2	2099.78 ± 117.33 [a]	9.71 ± 0.11 [a]	2357.01 ± 203.88 [a]	0.132 ± 0.013 [b]

Superscript letters designate significant differences among treatments ($p < 0.05$).

4. Discussion

4.1. The Effects of MPs on Soil Inorganic N and Mineralization Rate

Available N, including inorganic N (nitrate and ammonium) and dissolved organic N, is regarded as an important limiting factor for productivity in most terrestrial ecosystems. The results showed that no or little changes in net mineralization rate and inorganic N content in farmland soil were observed after two levels (1% and 5%) PE MPs addition in comparison to CK throughout the incubation period. This result was consistent with the observation of Blocker et al. [34], where soil inorganic N and mineralization rates after 1% low density polyethylene (LDPE) MPs addition were not different from control. A similar result was found by Ren et al. [35], who added 1% MPs to soil and no or little changes was shown in the soil inorganic N. In contrast to our results, an experiment from Liu et al. [19] indicated an increase of nitrate content at a very high MPs addition concentration (28%), but they also found no difference in ammonium content between different treatments. Chen et al. [20] also found that 2% polyactic acid addition had no significant effects on soil nutrients, including soil nitrate and ammonium contents.

The responses of soil inorganic N to MPs additions were observed in different studies, which may result from the experimental conditions, MP types and addition levels. Previous

studies have shown that MPs can alter soil physical and chemical properties, such as increasing soil porosity and changing the structure of soil aggregates [36–38], leading to alternation in soil microbial activity [39]. MPs addition levels were strongly correlated with microbial activity, affecting the release of soil N [40]. In comparison to the study by Liu et al. [19], we conducted incubation in a lower temperature (25 °C) and lower humidity (75%) without light, accounting for some of the differences. MPs breaking down to smaller plastics particles might accelerate UV-radiation under a light exposure condition [41,42]. Higher temperatures and higher humidity are beneficial for microbial activity [43]. In addition, the incubation term is an important reason to explain the differences in soil inorganic N. A short incubation term is so short that MPs could not decompose and display the effects on soil inorganic N.

4.2. The Effects of MPs on Soil DON and DOC

Soil soluble organic carbon and N are vital parts of soil organic matter and play an important role in soil nutrient biogeochemical cycling. Meanwhile, DON and DOC are sensitive indicators for the changes in soil quality [19,44]. Our results showed that soil DON and DOC content were significantly enhanced after MPs addition ($p < 0.05$). The tendencies along incubation time between DON and DOC were similar. Significant increases observed in soil DON and DOC at the first six days after MPs addition might due to the disturbance during soil and MPs mixing [19]. A decline tendency of DON and DOC from days 6 to 10 is also shown by the results of this study. Two reasons could be contributed to this phenomenon. On one hand, the consumption of resources and nutrients, such as soil organic matters, led to the decrease of microbial activity and quantity. On the other hand, the utilization of DON and DOC by microorganisms resulted in the decrease of soil DON and DOC contents. Plastic particles have a very high content of carbon [45], and this material will be decomposed slowly, contributing to a very high C: N ratio in soil. PE MPs could be utilized as C source by microorganisms and potentially enhanced microbial activities [46]. In turn, the increase in microbial activities further promoted soil organic matter decomposition, likely leading to the increases in soil DON and DOC contents from days 10 to the end of this incubation. However, it has to be emphasized that microbes are not only producers for soil DOC, but also a potential source of soil DOC [47] since the dead body of microorganisms was an important C source. Therefore, soil DON and DOC increased from days 15 to 30.

Currently, the impact of MPs on soil DON and DOC are inconsistent among different studies. For example, Liu et al. [19] indicated that the concentration of polypropylene MPs at 28% can promote the activity of hydrolase and increase the DOC content, a low concentration of 7% had no significant effect on soil DOC. In contrast to the studies conducted by Blocker et al. [34], the results suggested both PE (5%, *w/w*) and PLA (1%, *w/w*) additions did not have significant effects on DOC content. The reason for these differences in results may be attributed to the MPs type, morphology and their dose. Therefore, in order to better investigate the effects of MPs addition, further research about different MP types under various conditions is encouraged in the future.

4.3. The Effects of MPs on the Bacterial Community Diversity

These results reveal that the abundance of some bacterial families remains largely unaffected by PE addition throughout the incubation. Our results were consistent with the previous study conducted by Judy et al. [48], where no significant difference was observed in the bacterial community diversity under the 1% concentration addition with high-density polyethylene (HDPE), polyethylene terephthalate (PET), or polyvinyl chloride (PVC) MPs in comparison to the control. Notwithstanding, our study indicated a significant change in soil bacterial evenness index after MPs addition at a high concentration. This phenomenon may due to the number of dominant species in soil microorganisms at a high level of MPs addition, then an increase in the number of other species. Zhang et al. [31] suggested that MPs might promote the bacterial groups during the period of their own biodegradation,

resulting in an alternation in bacterial community structure. However, the interactions between MPs and microorganisms were complicated, largely depending on soil properties, MP types and their dose [31,49].

5. Conclusions

This study investigated the effects of MPs on soil available N by adding PE MPs to farmland soil. Responses of soil ammonium, nitrate and N mineralization rate to MPs addition were negligible. However, significant increases were observed in soil DOC and DON contents with the existence of MPs. The abundance of bacterial families was not affected by MPs, whereas a significant change in soil bacterial evenness was observed in a high level of MPs addition. According to the findings, we concluded that the occurrence of MPs poses a profound effect on soil DON rather than soil inorganic N. While MPs had no significant influence on bacterial richness, the number of each bacterial species tends to be homogeneous. The effects of MPs on soil N availability depended on experimental conditions, incubation term, MPs types and dose. With regard to a sustainable release of MPs into farmland ecosystem, it is urgently needed to address the alternations in soil biogeochemical processes affected by MPs in relation to their features and clarify the microbial mechanism.

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