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Compositional shifts in microbial communities in soils supplemented with iron oxide materials and inorganic fertilizer

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Abstract

Soil microbial communities play an important role in determining soil ecosystem health. However, the effect of chemicals added to the soil on the function and composition of the soil microbiome is not well known. This study evaluated the effects of magnetite materials and inorganic fertilizer on the microbial communities in the soils of both laboratory and field trials. The study used a culture-dependent technique using Czapek agar (CZA) and De Man, Rogosa, and Sharpe (MRS) agar media combined with a modified method for the assessment of soil microbial respiration to account for changes in the microbial community. Results performed with commercial vegetable soil in the laboratory and soils obtained from the field trial both showed an increase in soil respiration rates over time in response to fertilizer and iron oxide materials added to the soil. The iron oxide materials have been shown to have a stronger impact on the soil microbial communities compared to the fertilizer. In addition, the microbial population cultured on the MRS medium was considered to have an important role in total soil respiration. Further studies on the roles of different microbial communities in the soil as well as the combination of different analytical methods should be needed to improve our understanding of the relationship between soil microbial communities and changes in environmental conditions.

1. Introduction

The total soil CO_2 efflux is used as an indicator to evaluate soil respiration that reflects the activities of autotrophic roots (involving rhizosphere microorganisms) and heterotrophic microorganisms inhabiting the soil (Li et al., 2005; De Dato et al., 2017). Soil CO_2 emissions also represent the rate of soil carbon mineralization; hence, it is an important factor in soil management and aids in implementing interventions when the rate of carbon mineralization needs to be reduced (Jónsson et al., 2017). Heterotrophic respiration by soil microorganisms contributes the most to the total soil respiration (Li et al., 2006). It varies with the type of ecosystem and is highly dependent on biotic and abiotic factors (De Dato et al., 2017). Soil respiration has also been used to evaluate changes in soil microbial communities in response to variations in environmental conditions in various geological regions (Li et al., 2006; Wei et al., 2015).

The health of an ecosystem is highly dependent on its microbial community, which includes biomass, activities and interactions among the microorganisms (Madrova et al., 2018; Rogiers et al., 2021). Apart from geographical and natural factors, anthropogenic factors such as pollutants released into the environment also influence the activity and function of soil microbiota. Several studies have revealed that exposure to heavy metals leads to an adaptation mechanism involving changes in the composition, activities, and network interactions of soil microbial communities. The responses of each microorganism to different heavy metal pollutants are varied, and the highest resistance capability is observed in archaea (Li et al., 2017). Long-term exposure to toxic elements, such as Pb, Zn, As, and Cd, in soils, induces changes in bacterial composition, but not the bacterial diversity and total biomass. Additionally, in secondary disturbance experiments, an increase in Cd levels led to a decrease in soil bacterial diversity,

while soil respiration increased with an increase in bacterial population (Madrova et al., 2018). Another study of microbial communities in river sediments with a metal-contaminated gradient revealed that microbial composition was significantly correlated with organic matter and metals (Bouskill et al., 2010). On the other hand, adding organic fertilizers to soils could increase soil respiration and soil organic carbon contents (Yang et al., 2018; Huang et al., 2021; Hernandez et al., 2021). Inorganic fertilizers added to soils could also induce effects on soil respiration and microbial activities but with significantly lower effect levels than organic fertilizers (Iovieno et al., 2009; Li-mei et al., 2011; Yang et al., 2017; Hernandez et al., 2021).

The emergence of nanotechnology and its widespread application has increased the risk of environmental contamination with nanomaterials, especially metal nanoparticles (Rajput et al., 2018). Nanomaterials can have disparate properties from their core metals with regard to their size and form. Although there have been only a few studies on the effects of metal nanomaterials, particularly nanoparticles, on ecosystems, some have divulged the influence of metal nanoparticles on the structure and function of soil microbial communities. Silver nanoparticles (AgNPs) impact the structure and resilience of microbial communities (Grün et al., 2019) and alter the structure and function of bacterial communities in unplanted and cucumber-planted soils (Zhang et al., 2020). A study with metal oxide nanoparticles indicated that CuO and Fe_3O_4 nanoparticles affect the hydrolytic activity and composition of soil bacterial communities (Frenk et al., 2013).

Culture-dependent methods have traditionally been used to analyze soil microbial communities, and soil respiration rate is used to evaluate soil microbial properties (Hill et al., 2000). Due to limitations in detecting and identifying microorganisms, only limited progress has been made thus far toward understanding microbial diversity (Vitorino and Bessa, 2018). It is estimated that ~99% of microorganisms in nature are typically not culturable using standard techniques (Hill et al., 2000). Hence, there is a great demand for more comprehensive approaches to study microbial communities in diverse environments (Su et al., 2012). Therefore, culture-dependent methods are combined with culture-independent methods to study the correlation between the structure and function of microbial communities and environmental factors (Stefani et al., 2015; Li et al., 2019).

In this study, we report a simple setup for measuring changes in the microbial community of a commercial vegetable-planting soil and a soil from the phyto-Fenton field trial conducted in Ha Tinh province, Vietnam. The method is a simplified modification of a preexisting procedure to assess soil microbial respiration (Moebius-Clune, 2016). A culture-dependent technique using Czapek agar (CZA) and De Man, Rogosa, and Sharpe (MRS) agar media was implemented to measure changes in microbial numbers and to find a correlation between microbial populations and soil respiration rates.

2. Materials and methods

2.1. Materials

The iron oxide nanomaterials used in this study were nano- and micro-magnetite (Fe_3O_4) (CAS 1317-61-9; Sigma-Aldrich, Germany). The soil was Tribat clean soil (Saigon Xanh Biotechnology Co. Ltd., Saigon, Vietnam), which was pretreated for pathogens and preconditioned with minimal nutrients and minerals necessary for plant growth (total organic compounds 24.91%, humic acid 14.45%, total nitrogen 0.9%, K_2O 0.73%, P_2O_5 0.3%, cation exchange capacity of 44.69 cmol kg^{-1}, and other necessary medium- and micro-elements in chelated forms). FJ30-10-10 inorganic fertilizer (total nitrogen 30%, P_2O_5 10%, and K_2O 10%; Fuji Bio Co. Ltd., Vinh Long, Vietnam) was used in this study. Pea (*Pisum sativum*) seeds (Rang Dong Co. Ltd., Hanoi, Vietnam) were cleaned and packed in closed tin bags.

The soils from the field trial were collected directly from the trial site in Cam Binh town, Cam Xuyen district, Ha Tinh province, Vietnam (18°17'29.5'' N; 105°57'31.5'' E). Samples were collected monthly starting from December 2020. Each soil sample was a mixture of five sampling points around 10 cm below the soil surface in each experimental plot. After removing small stones and plant debris, samples were air-dried at 24°C, and well ground before being analyzed. The initial properties of soil were pH 7.54, total organic carbon 0.47%, cation exchange capacity of 13.37 cmol kg^{-1}, total nitrogen 0.08%, and P_2O_5 0.07%.

The microbial media used in this study were CZA medium (saccharose 30 g L^{-1}, sodium nitrate 2 g L^{-1}, dipotassium phosphate 1 g L^{-1}, magnesium sulfate 0.5 g L^{-1}, potassium chloride 0.5 g L^{-1}, ferrous sulfate 0.01 g L^{-1}, and agarose 20 g L^{-1}; final pH 7.3±0.2) and MRS agar medium (Merck, Germany). CZA medium is a growth medium used for propagating fungi and other aerobic organisms in the laboratory and is recommended for use in qualitative procedures for cultivating saprophytic fungi, soil bacteria, and other microorganisms. MRS agar medium is optimized and recommended for the isolation and growth of all species of the genus *Lactobacillus*.

2.2. Experimental design for the assessment of soil CO_2 emission

The experimental design is illustrated in Figure 1. The soil samples were pre-dried naturally to a constant weight, and 20 g of dried sample was added and evenly coated on the bottom of a glass jar. A plastic tripod was placed in the jar at the center of the top soil layer. The distance between the soil and tripod surfaces was about 1 cm. Next, a glass beaker containing 10 ml of 0.5 M KOH solution (CAS 1310-58-3; Sigma-Aldrich, Germany) was placed on the tripod. Sterile distilled water (7 ml) was gently pipetted by placing the pipette tip on the inner wall of the jar. The mouth of the jar was covered with a lid and sealed with a vaseline layer to ensure that there was no influx or efflux of gas. Finally, the glass jars were placed in a well-ventilated area with natural light.

Following the replenishment of soil moisture, soil microorganisms began to perform respiratory and metabolic processes.

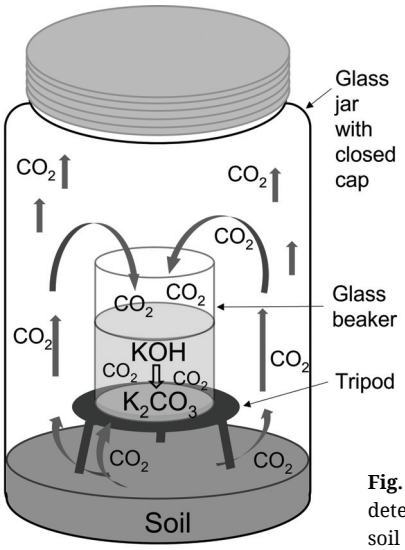

Fig. 1. Experimental design for detecting CO_2 emission from the soil due to microbial respiration

As a result, CO_2, which is a product of microbial respiration, escaped from the soil and was partially absorbed by the KOH solution to create K_2CO_3, thus gradually decreasing the pH of the solution. The balanced equation of this reaction is given below:

$$2KOH + CO_2 \leftrightarrow K_2CO_3 + H_2O \quad (1)$$

The solution was considered saturated when all the KOH was converted into K_2CO_3, i.e., 10 ml 0.5 M KOH was converted into 0.25 M K_2CO_3. The equilibrium of CO_3^{2-} in H_2O can be expressed using the following equation:

$$CO_3^{2-} + H_2O \leftrightarrow HCO_3^- + OH^- \quad (2)$$

Since the CO_3^{2-} acts as a base, the K_b is expressed as

$$K_b = [HCO_3^-][OH^-]/[CO_3^{2-}] = K_w/K_a \quad (3)$$

where K_w (10^{-14}) and K_a (5.6×10^{-11}) are the acid dissociation constants of H_2O and H_2CO_3, respectively. K_b was then calculated to be 1.79×10^4. The dissociation of OH^- from H_2O could be neglected under this condition, and it was valid to assume that $[OH^-] = [HCO_3^-]$. Furthermore, there was a small amount of CO_3^{2-} that converted to HCO_3^-, so $[CO_3^{2-}]$ was assumed to remain at 0.25 M. Based on these assumptions, $[OH^-]$ can be calculated from the equation for K_b as follows:

$$[OH^-] = [HCO_3^-] = ((10^{-14}) \times 0.25/(5.6 \times 10^{-11}))^{1/2} = 6.69 \times 10^{-3} \quad (4)$$

The pH of 0.25 M K_2CO_3 solution was calculated as follows:

$$pH_{K2CO3} = 14 - pOH = 14 - (-\log[OH^-]) = 11.83 \quad (5)$$

2.3. Experimental conditions during the soil microbial study

The initial pH and moisture of the Tribat clean soil were 7 and 28%, respectively, measured using a soil tester (MS04; Sonkir, Hanoi, Vietnam). The soil was pre-dried naturally to < 20% moisture content. Experiments were conducted in 24 cm (length) × 24 cm (width) × 20 cm (height) plastic pots under two treatment conditions: natural and mixed soils. The mixed soil contained magnetite nanomaterials at a concentration of 25 mg kg^{-1} of soil. To mimic natural conditions, each pot containing 2 kg of soil was seeded with 12–15 pea (*Pisum sativum*) seeds sown in rows with equal spacing. Same-sized seeds were pre-selected and soaked in distilled water for 5 h before sowing. Plant development in response to each experimental condition was also observed but will not be reported here. The experimental pots were placed outdoors in a 1.8 m (length) × 1.2 m (width) × 1.4 m (height) wooden frame box with walls and roof covered with 0.5 mm thick transparent nylon sheets. Each treatment was conducted in triplicates. The experimental conditions were as follows: ambient temperature ranged from 19°C to 22°C at night and 25°C to 29°C during the day, with 80–90% relative humidity and 1500–1600 lx natural sunlight. The soil moisture during experiments was maintained at approximately 80%.

In the field trial in Ha Tinh province, magnetite micromaterials were supplemented to the soils to initiate a Phyto-Fenton process for the removal of pesticide residues. The Phyto-Fenton process is a process created by combining endogenous hydrogen peroxide produced by plants and iron oxide materials as a catalyst for the mass production of hydroxyl radicals (·OH) which are strong oxidizing radicals to decompose persistent organic compounds in soil and water environments (Inagaki et al., 2016). The pesticides to be removed in this trial were dichlorodiphenyltrichloroethane and its persistent metabolites, dichlorodiphenyldichloroethane, and dichlorodiphenyldichloroethylene. In the trial, four different treatment conditions were carried out which are original soil, soil supplemented with micromaterials at 200 mg kg^{-1}, soil supplemented with fertilizer at 62.5 mg kg^{-1}, and soil supplemented with both micromaterials at 200 mg kg^{-1} and fertilizer at 62.5 mg kg^{-1}.

2.4. Data collection and analysis

The jars were opened, and the pH of the solution in the beaker was measured at the day 2, 4 or 6 using a pH meter (Hanna pH-211, Sigma-Aldrich, Germany). The relative pH variation is an indicator of the respiration rate of soil microorganisms, which depends on the structure and composition of the soil microbial population. The relative pH variation is determined using the following formula:

$$S_{pH}\ (\%) = ((pH_{KOH} - pH_t) \times 100)/(pH_{KOH} - pH_{K2CO3}) = ((pH_{KOH} - pH_t) \times 100)/(pH_{KOH} - 11.83) \quad (6)$$

where S_{pH} is the relative change in the pH of the solution due to the conversion of KOH to K_2CO_3; pH_{KOH} is the pH of the original KOH solution; pH_{K2CO3} is the pH of the saturated solution; pH_t is the pH of the solution obtained at the day 2, 4, or 6. Each measurement condition was repeated at least thrice. Once the jar was opened, the jar was not considered for further analyses.

Microbial count experiments were conducted using the traditional plate count method. In brief, soil samples were dried at room temperature, and then 1 g of each sample was scaled and ground. Next, the samples were diluted in distilled water to the concentration of 10^{-5}, then 50 µl of dilution was spread on Petri dishes containing agar medium, and incubated at 35–37°C for

2 days in an incubator (IF110, Memmert, USA). Finally, microbial colonies that grew on the dishes were counted to calculate the microbial population expressed as CFU g^{-1} (colony-forming unit per gram of sample).

The collected data were imported into excel files, and statistical analysis of the data was performed using one-way ANOVA. In addition, Fisher's LSD tests were used to compare the means of the treatments. All analyses were conducted using the statistical package StatPlus LE Build 7.3.0.0 for Windows (AnalystSoft Inc., Walnut, CA, USA). The significance level was set at $p < 0.05$.

2.5. Validation of the experimental design

The original soil, unsupplemented Tribat clean soil, was subjected to the experiment for the assessment of CO_2 emission from soil microbial respiration by following the procedure mentioned in sub-section 2.2. Jars were opened at regular time intervals of 2, 4, and 6 days to measure the pH of the solution. The results shown in Figure 2 indicate that the experimental setup works, and the increasing CO_2 generated from soil microbial activities could be detected in this experiment. According to the results obtained, a time interval of 4 days was chosen for the subsequent experiments to measure the relative pH change in the KOH solution.

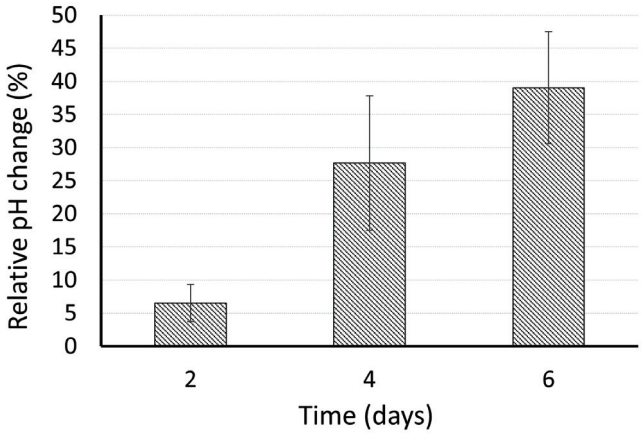

Fig. 2. Relative changes in the pH of the KOH solutions with time in the experimental setup to detect increasing CO_2 generation by soil microbial respiration

3. Results

3.1. Increase in soil respiration in correlation with the increase in soil microbial population

The commercial clean soil was used to observe the differences in soil respiration with a change in the soil microbial population in 30 days. Figure 3 shows the increase in the microbial populations detected on CZA and MRS agar media. MRS population showed a faster developmental rate during the first 7 days, followed by a stable growth period for both populations, and then the CZA population increased. Furthermore, the relative pH changes increased with an increase in microbial populations; however, the correlation was not linear. The stable period may result from increasing interactions within the larger microbial population and restructuring of the microbial community to achieve a new balance. As different soil microorganisms may have disparate roles in the microbial community, their respiration rates may differ, as shown by a slight decline in soil microbial respiration during the time from days 17 to 30 of the experiment.

3.2. Iron oxide nanoparticles and inorganic fertilizer induce compositional shifts in the soil microbial community

This study explored the effects of magnetite nanomaterial- and inorganic fertilizer-supplemented soil on the soil microbial community. The CZA populations in all treatments showed similar growth trends (Fig. 4a). The microbial populations in the control and nanomaterial-supplemented soil showed a minor difference in the first 17 days and no significant difference during the period of days 17 to 30. The microbial population in fertilizer-supplemented soil showed a similar growth trend as that

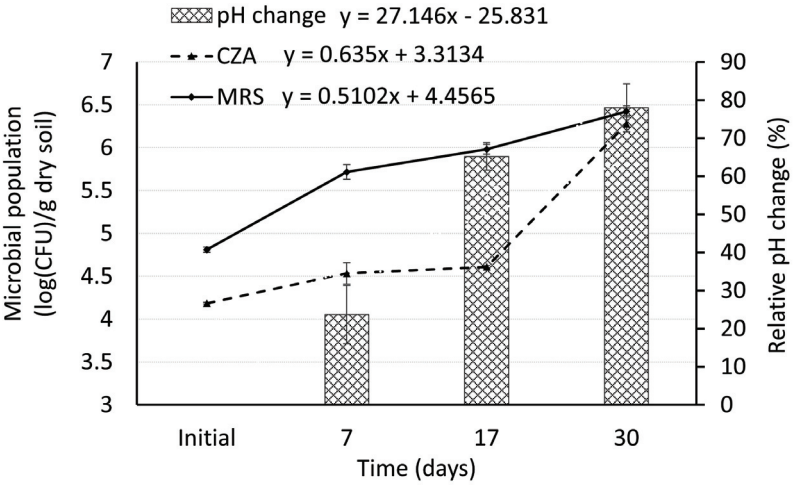

Fig. 3. Soil respiration changes in response to the changes in soil microbial populations. CZA and MRS denote the soil microbial population detected by Czapek agar and De Man, Rogosa, and Sharpe (MRS) agar media, respectively

in the control and nanomaterial-supplemented groups, and microbial counts were lower during the whole experiment. Furthermore, the MRS populations (Fig. 4b) showed similar growth trends with no significant differences during the first 17 days. In the nanomaterial-supplemented soil, there was a significant increase in growth rate during the latter days and peaked on day 30, which was the highest microbial count followed by those of the control and fertilizer-supplemented soil.

The relative pH changes in KOH solution in accordance with the changes in soil microbial populations were the most in the fertilizer-supplemented soil, indicating that this sample had the highest respiration rate (Fig. 4c). Microbial respiration in nanomaterial-supplemented soil indicated a faster respiration rate than that of the control soil during the first 7 days, followed by a period of stability before increasing rapidly and reaching the same value as the fertilizer-supplemented soil on day 30. The respiration rate in the control soil increased gradually, peaking on day 17 and then stabilizing for the remainder of the experimental period.

The shifts in soil microbial composition have been assessed for the soils in a field trial conducted in Ha Tinh province, Vietnam from December 2020 to April 2021. Results indicated that the microbial populations in the CZA medium (Fig. 5a) grew faster in soils supplemented with either fertilizer or fertilizer plus micromaterials during the first month, before entering a steady period with no significant increase in the next month. The CZA population of the control case showed a steady growth and reached a maximum after 2 months, which was significantly higher compared to the other cases. The CZA population of micromaterial-supplemented soil also showed a steady growth with a lower rate compared to the control and reach a similar value to the two cases where soils were supplemented with either fertilizer or fertilizer plus micromaterials after 2 months. The microbial populations on the MRS medium (Fig. 5b), meanwhile, indicated no increases during the first month except for the case where soil was mixed with both fertilizer and micromaterials. The MRS populations of the control and the two cases where soils were supplemented with either fertilizer or fertilizer plus micromaterials reached a similar after 2 months. The MRS population on micromaterial-supplemented soil was the lowest with a significant difference from the other cases at the point of 2 months.

Observation on the data on the soil respiration (Fig. 5c) indicate similar growth rates in all treatments during the first month of the trial with a slight difference in fertilizer-supplemented soil. At the 2-months point, data showed significant differences with the highest and lowest values belong to the control and micromaterial-supplemented soil, respectively. This shows a correlation in the data for microbial population and soil respiration since the observation at the 2-months point indicated the total microbial population was the highest for the control soil and the lowest for micromaterial-supplemented soil.

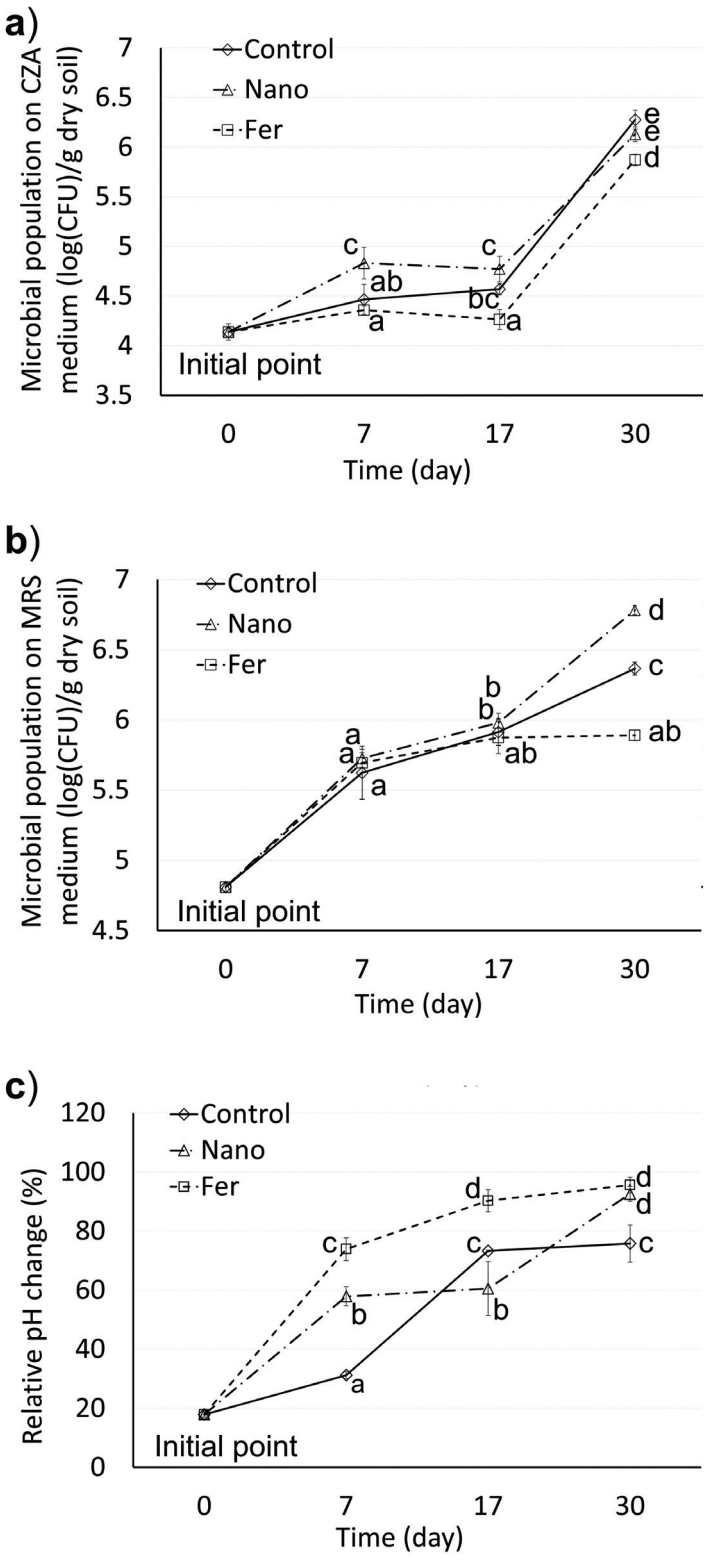

Fig. 4. Soil respiration changes in accordance with the changes in soil microbial populations due to magnetite nanomaterials and fertilizer supplementation in soil. Control, Nano, and Fer denote the original soil without any supplementation, magnetite nanomaterials-supplemented soil at the concentration of 25 mg kg^{-1} of dry soil, and fertilizer-supplemented soil at the concentration of 5 g kg^{-1} of dry soil, respectively. Different letters (a to e) represent significant statistical differences (Fisher's LSD, $p < 0.05$)

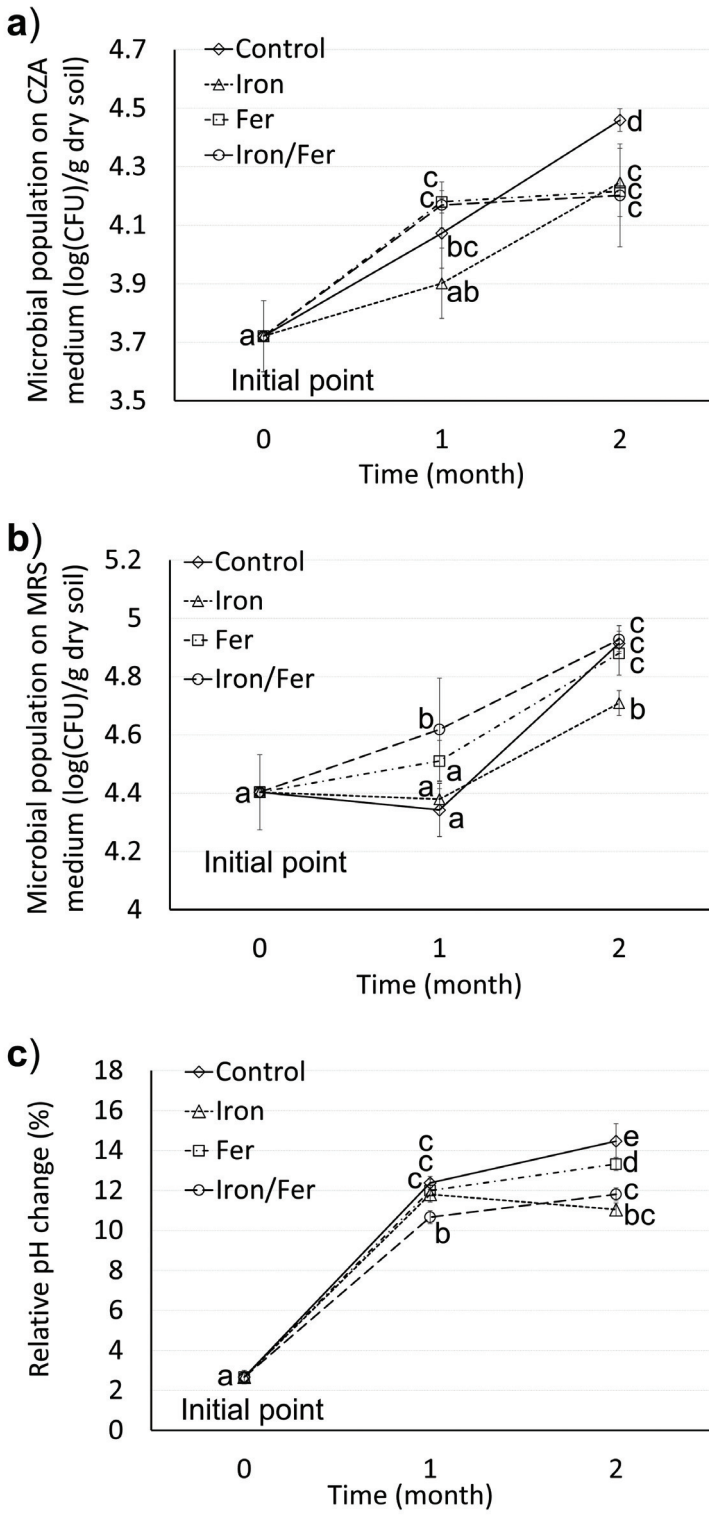

Fig. 5. Soil respiration changes in accordance with the changes in microbial populations in soils from the field trial in Ha Tinh province, Vietnam. Control, Iron, Fer, and Iron/Fer represent the original soil without supplementation of micro-materials or fertilizer, micro-materials-supplemented soil at the concentration of 200 mg kg^{-1} of dry soil, fertilizer-supplemented soil at the concentration of 62.5 mg kg^{-1} of dry soil, and soil supplemented with micro-materials at 200 mg kg^{-1} and fertilizer at 62.5 mg kg^{-1} of dry soil, respectively. Different letters (a to e) represent significant statistical differences (Fisher's LSD, $p < 0.05$)

4. Discussion

Organic and inorganic fertilizers increase soil respiration and microbial activity (Iovieno et al., 2009; Yang et al., 2018; Huang et al., 2021). The results on the growth of microbial population and soil respiration in fertilizer-supplemented soil were consistent with the findings of previous reports; the soil respiration significantly increased, while the growth rate of the microbial population was similar to that of the control and nanomaterial-supplemented soil samples (Figure 4). Interestingly, during the period from day 17 to day 30, the rapid increase in the rate of soil microbial respiration seemed to correspond to the rapid increase in the MRS microbial population in the nanomaterial-supplemented soil. Furthermore, the stability of microbial respiration corresponded to the stability of the MRS population in the fertilizer-supplemented soil during this period. This suggests that the MRS microbial community may have significantly contributed to the soil microbial respiration in this study.

In the study with soils from the field trial, it is interesting that at the 1-month point both the CZA and MRS populations of soil supplemented with both fertilizer and micro-materials were significantly higher compared to those in the other cases (Fig. 5a and b). The microbial respiration, meanwhile, was the lowest. This suggested there were changes in microbial activities leading to changes in soil respiration rate. This once again shows consistent with findings in previous reports on the effects of inorganic and organic fertilizers on soil microbial community (Iovieno et al., 2009; Yang et al., 2018; Huang et al., 2021). In the case of micromaterial-supplemeted soil, although both the CZA and MRS populations showed significantly low compared to those in the other cases at the 1-month point, the respiration rate showed no significant difference (Fig. 5c). At the 2-months point, while the MRS population was the lowest, the respiration rate did not show a significant difference from the case of soil supplemented with both micromaterials and fertilizer.

All results suggested that there are shifts in the composition and activity of the soil microbial communities in response to different chemical factors supplemented to the soils. Thru this study, it seems that the magnetite materials induced a stronger effect on soil microbiota compared to that of the fertilizer. It was expressed thru an increase in soil microbial activities by increasing the respiration rate when observing at the 1-month point, in which there could have a significant role of MRS population.

Different microorganisms could have different responses to each factor introduced into the soil and thus induce different shifts in the composition of the microbiome. Azarbad et al. (2015) observed a significant impact of metal pollution on soil bacterial community structure corresponding to changes in the relative abundance of specific bacterial taxa, including *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Firmicutes*, *Planctomycetes,* and *Proteobacteria.* A study on metal-contaminated river sedi-

ments from the Montana Superfund site showed a stable metal-tolerant community at the most contaminated sites with *Beta* and *Gammaproteobacteria* found predominantly from DGGE-extracted bands (Bouskill et al., 2010). Bacterial communities of the less contaminated soil were found more homogeneous compared to that of the highly contaminated soil in a study with long-term contaminated soils with toxic elements including Pb, Zn, As, and Cd (Madrova et al., 2018). The study also suggested that, among soil bacterial communities, *Actinobacteria* can particularly withstand toxic elements. On the other hand, soil type and composition are also the factors influencing the soil microbial activities and structure. Li et al. (2005) found reductions of both soil CO_2 efflux and microbial biomass in soils with litter and root exclusion that suggests a critical role of carbon input from aboveground to the soil microbial community and ecosystem carbon cycling. Soil texture, particularly the grain size distribution of soils showed a correlation with the toxicity of silver nanoparticles on soil microbial communities expressed through several biological parameters of soils including the microbial biomass, the abundance of bacteria, the enzymatic activity, and marker genes for selected processes (Grün et al., 2019). More works should be needed to elucidate the correlation between soil respiration and microbial structure and function in soil.

Agricultural activities, such as the use of pesticides, have led to the contamination of soils (Braunschweig et al., 2013; Pentráková et al., 2013). A wide range of microorganisms exists in agricultural soils, some of which play a role in pesticide degradation (Yao et al., 2006). Hence, iron oxides have been extensively studied as potential agents to enhance the degradation process for in situ remediation of contaminated soils (Braunschweig et al., 2013). This iron-based remediation involves the oxidative and reductive transformation of organic contaminants, such as chlorinated hydrocarbons (Borch et al., 2010). Zero-valent iron (ZVI) is a good example of an electron donor for transforming chlorinated organic compounds as well as for aiding microbial growth (Dombek et al., 2001). Iron compounds interact with soil microorganisms in various ways, such as acting as electron acceptors for microbial anaerobic respiration under anoxic conditions (Fortin and Langley, 2005). Direct contact between microorganisms and the solid iron oxide can form conductive cellular nanowires, creating a bridge for reducing iron oxides by the microorganisms (such as *Geobacter* and *Shewanella*) (Weber et al., 2006). Another iron reduction scheme involves iron-reducing bacteria (IRB) in the presence of humic acids, which are abundant in soils and regarded as electron acceptors for organic matter oxidation (i.e., detoxification of environmental contaminants); IRB can pass electrons from humic acids to insoluble iron oxides, which behave like terminal electron acceptors (Lovley et al., 1996). This electron shuttling could be the underlying mechanism accelerating contaminant degradation in humic acid-rich soils.

Nanosized iron oxides, which can diffuse into intercellular spaces between different microorganisms, can act as electron mediators. Kato et al. (2012) demonstrated the facilitation of electron transfer between *Geobacter sulfurreducens* and *Thiobacillus denitrificans* with nanosized magnetite. Although Park et al. (2010) studied the impact of nanosized ZVI (nZVI) on IRB metabolism and found that nZVI did not affect the microbial population, recent studies have shown that iron oxides can shift soil microbial composition. For example, *Proteobacteria*, *Firmicutes*, and *Actinobacteria* were the dominant bacterial phyla in iron-abundant soils owing to their tolerance for C-substrate deficiencies (Jeewani et al., 2021). This study clearly indicates that both nanosized and microsized magnetite added to soils can place an impact on the composition of soil microbial community expressed through changes in the abundances of microbial populations on CZA and MRS culture media and total soil microbial respiration. Results from laboratory samples with nano-magnetite and field-trial samples with micro-magnetite both suggest a critical role of MRS populations in the changes in soil respiration rate. The study, however, could not give inside into the mechanism of changes in the soil microbial community's composition and respiration since it might need more investigations into the activities and adaptive capacity of different microbial communities in soils.

Triangular interactions among microorganisms, iron oxides, and organic carbon in soils have not received much attention (Pentráková et al., 2013). In iron-rich soils, organic matter degradation may be prevented (i.e., organic carbon preservation) due to multiple reactions with iron oxides, including adsorption, aggregation, and coagulation (Herndon et al., 2017). However, biological decomposition of organic matter in soils results in i) the release of electrons or other reducing agents into the surrounding soil, which lowers redox, increasing the solubility of ferrous iron, and ii) the production of organic acids and other breakdown products, which could lead to the formation of soluble Fe-organic complexes (Fortin and Langley, 2005). When iron oxide is reactive, it may catalyze the production of reactive oxygen species and negatively affect microbial activity (Cai et al., 2022). It is still unclear whether the addition of iron oxide to soils, especially at the nanometric scale, can promote or hinder the activity of microorganisms. Hence, the variations in the microbial respiration response still need to be assessed in soils mixed with nanosized iron oxides.

5. Conclusions

In this study, we report the use of a simple culture-dependent technique and soil respiration assessment method to study the changes in the microbial community in response to magnetite materials and inorganic fertilizer supplementation to the soil. Both the iron oxide materials and fertilizer impact the soil microbial community by increasing the respiration rate and changing the community structure by altering the microbial population cultured on the MRS medium. This suggests an important role of the MRS population in the changes in total soil microbial respiration. Compared between samples, it seems that the impact of magnetite materials on the soil microbial community is stronger than the inorganic fertilizer added to the soils. The soil microbial community has a highly complex structure with many prokaryotic and eukaryotic microorganisms, each of which can respond differently to certain factors in the soil. Therefore, along with developing more comprehensive approaches to study soil

microorganisms, more studies on soil microbial communities are needed to gain a deeper understanding of how the microbial communities respond to changing environmental conditions and implement efficient interventions for soil management.

Acknowledgments

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