Organic soil amendments using vermicomposts under inoculation of N$_2$-fixing bacteria for sustainable rice production

Mehdi Ghadimi$^1$, Alireza Sirousmehr$^1$, Mohammad Hossein Ansari$^2$ and Ahmad Ghanbari$^1$

$^1$Department of Agronomy, University of Zabol, Zabol, Sistan-o-Baluchestan, Iran
$^2$Department of Agronomy, Rashht Branch, Islamic Azad University, Rashht, Iran

ABSTRACT

Organic and biological fertilizers are considered as a very important source of plant nutrients. A field experiment was conducted during 2017–2018 in paddy soil to investigate the effect of vermicomposting of cattle manure mixture with Azolla and rice straw on soil microbial activity, nutrient uptake, and grain yield under inoculation of N$_2$-fixing bacteria. Experimental factors consisted of organic amendments at six levels (vermicomposts prepared from manure (VM); manure + rice straw (VRM); manure + Azolla mixture (VAM); manure + rice straw + Azolla mixture (VRAM); raw manure without vermicomposting (M), and a control) and N$_2$-fixing bacteria at three levels (Azotobacter chroococcum, Azospirillum brasilence, and non–inoculation). The results showed that, vermicompost treatments compared to control and raw manure significantly increased the number and biomass—C of soil microorganisms, urease activity, number of tillers hill$^{-1}$, phosphorus (P) and potassium (K) uptake, and grain and protein yield. Inoculation of plants with N$_2$-fixing bacteria, especially Azotobacter increased the efficiency of organic amendments, so that the maximum urease activity, soil microbial activity, P and N uptake, and grain yield (4,667 (2017) and 5,081 (2018) kg/h) were observed in vermicompost treatments containing Azolla (VAM and VRAM) under inoculation with Azotobacter. The results of the study suggested that, using an organic source along with inoculation with appropriate N$_2$-fixing bacteria for vermicompost has a great effect on enzyme activity, soil biology, nutrient uptake and grain yield has a synergistic interaction on agronomic traits under flooded conditions. Therefore, this nutrient method can be used as one of the nutrient management strategies in the sustainable rice production.

INTRODUCTION

The use of organic and biologic fertilizers in rice production is one of the most important methods used for soil fertility in a sustainable system (Wang et al., 2015; Amanullah, 2016; Chen et al., 2018). Among various organic fertilizers, vermicompost superiority...
has been proved in maintaining the long-term soil fertility (Lim et al., 2012; El-Haddad et al., 2014; Guridi et al., 2017; Sharma & Garg, 2018). Actually, soil amendment with vermicompost is an agronomically interesting practice as well as an attractive waste management strategy (Ludibeth, Marina & Vicenta, 2012; Baghbani-Arani & Modarres-Sanavy, 2017). Production of vermicompost as an easy and nature-friendly technology is a semi-aerobic process carried out by a specific group of earthworms (Eisenia fetida) and some soil microorganisms, especially bacteria and actinomists used for production of organic fertilizers from the waste and stabilization of these materials (Mahanta et al., 2012; Lim, Lee & Wu, 2016). As a result, stabilization reduces the environmental problems associated with manure (such as salinity) by transforming it into safer and more stabilized material for soil amendment (Mahanta et al., 2012). In addition to less time for bioconversion, the presence of higher content of minerals, hormones, enzymes, humic acids and bioavailable form of nutrients has been reported for vermicompost (Campitelli, Velasco & Ceppi, 2012; El-Haddad et al., 2014; Khan, 2018).

Vermicompost contains nutrients in such forms that are readily available to the crops, such as nitrates, exchangeable P, soluble K, iron (Fe), calcium (Ca), magnesium (Mg), etc. (Tejada & González, 2009; Ludibeth, Marina & Vicenta, 2012). Guridi et al. (2017) reported that, vermicomposts contain biologically active substances such as plant growth regulators, and have great potential in maintaining the soil fertility. If vermicomposts are integrated in nutrient management in agricultural fields, the costs of crop production may be reduced significantly (Lim, Lee & Wu, 2016; Sharma & Garg, 2018). On the other hand, some composts and vermicomposts (especially those made from chaff of wheat or rice straw straw) with high value of carbon-to-nitrogen (C/N) may increase the time of bioconversion of the crop or stimulate N fertilization in the soil, and face the plant with N deficiency in some growth stages (Zhu et al., 2013; Li et al., 2015; Eo & Park, 2019).

The use of *Azolla pinnata* (a free-floating weed) is a solution for increasing the N contents and vermicomposts. In terms of nutrients, the amount of *Azolla* nutrient has been shown to vary in different periods of time and has an average of 5.3% N, 8.3% K and 0.6% Mg, and is free from lead, mercury or arsenic (Sreenivasa, 2012). It has been reported that, vermicomposting of the straw is mixed with *Azolla* along with cattle dung to obtain a better quality crop suitable for agricultural applications (Arora & Kaur, 2019).

The use of bacteria with the capability for Biological N Fixation (BNF), such as *Azospirillum*, *Herbaspirillum*, and *Azotobacter*, as an effective strategy is another solution for increasing N amount in paddy soil, under nutrient management for production of safer and cheaper rice (Ladha & Reddy, 2003; Sanati et al., 2011; Zhang et al., 2017). In this regard, Bhattacharjee, Singh & Mukhopadhyay (2008) showed an increase in host-plant N content, up to 30–45 mg of N plant$^{-1}$ (6-week-old seedlings) resulted from N$_2$-fixing. This group of bacteria, in addition to the N$_2$-fixing capability in a cooperative way, contributes in solving the nutrients such as P, K, and iron (Fe), and also has the capability for producing Phytohormones, Vitamins and Siderophores (Zhang et al., 2018). Due to their positive effects on plant growth stimulation, these microorganisms are called as Plant Growth Promoting Rhizobacteria (PGPR) (Wu et al., 2005). Mahanta et al. (2012) revealed the superior performance of PGPR in increasing rice growth and grain yield and
Table 1 Results of physio-chemical analysis of the experimental soil.

| Year | Sand (%) | Silt (%) | Clay (%) | EC (dS/m) | pH | Organic Carbon (%) | N (%) | P (mg/kg) | K (mg/kg) | Zn (mg/kg) | Fe (mg/kg) | Mn (mg/kg) |
|------|----------|----------|----------|-----------|-----|--------------------|-------|-----------|-----------|------------|------------|------------|
| 2017 | 29.8 | 26.3 | 43.9 | 1.05 | 7.6 | 0.75 | 0.71 | 15.8 | 164 | 0.89 | 4.41 | 6.21 |
| 2018 | 28.5 | 32.2 | 39.3 | 1.24 | 7.1 | 0.86 | 0.66 | 12.4 | 194 | 1.23 | 5.82 | 7.51 |

improving soil health in addition to saving 40–80 kg N ha\(^{-1}\). In this regard, there is great interest in exploring the diversity of PGPR as substitutes for some chemical agricultural inputs (Rodrigues, Ladeira & Arrobasp, 2018). Generally, the N-transformation processes in the rice rhizosphere include N mineralization, denitrification, N fixation, and ammonia volatilization. Microbial-mediated mineralization and BNF are very important to the level of available N content in the soil and N uptake by rice (Pattanaik et al., 1999; Nayak, Babu & Adhya, 2007; Kumar, Swain & Bhadoria, 2018; Zhang et al., 2018), so an increase in the abundance of microbial populations could result in a faster N mineralization rate and increased N availability for plants (Li et al., 2015; Chen et al., 2017; Khan, 2018).

In Iran, rice is the second source for supplying food after wheat, with an annual consumption of 39.4 kg per person (Ashoori et al., 2018). Since Iran is located in a warm and dry region, its soils have low organic matter, and it is noteworthy that, the organic matter of the soil as the most important factor in crop yield ranks after the water. On the other hand, 20 million tons of manure is produced by animals per annum in Iran. The rate of compost and vermicompost production is negligible compared to manure. Efforts have been taken in recent years to produce more vermicompost, but its application in farms, especially in rice farms has not been increased remarkably (Rezaei, 2013; Taheri Rahimabadi, Ansari & Razavi Nematiollahi, 2018). In the present study, cow manure vermicomposting process was done in different mixtures with Azolla and rice straw under inoculation of N\(_2\)-fixing bacteria aimed at improving the agronomic value of the vermicomposts on promoting biological activity of paddy soil, nutrient uptake and grain yield of rice.

**MATERIALS AND METHODS**

**Experimental site and plant growth conditions**

The field experiment was conducted on clay-loam soil at the Rice Research Institute Farm of Rasht, Guilan province, Iran during 2017–2018. The area is located at 37°22N latitude and 49°63E longitude and 15 m above the sea level. To simplify the comparison of the growing season weather, the monthly total precipitation and temperature were considered from May to September at the Rasht Agricultural Research Farm (Figs. 1A and 1B). To determine soil characteristics, soil sampling was performed before the experiment. To do this, field soil sampling was done from the depth of 0–30 cm in 8 spots. Then, the collected samples were sent to the laboratory to determine soil texture and chemical composition. Properties of experimental soil samples are given in Table 1.
Experimental design and treatments

The experiments were carried out in a factorial trial based on Randomized Complete Block Design (RCBD) with three replications. Experimental factors consisted of organic amendments at six levels (M, VM, VRM, VAM, VRAM 10 t/hm², and a control) and N₂-fixing bacteria at three levels (Azotobacter chroococcum, Azospirillum brasilence, and non-inoculation). The organic amendments that included of manure and vermicomposts prepared from manure alone or mixed with different materials are presented in Table 2.

Preparation of vermicompost

In the present study, rice straw was collected from paddy fields and dried and chopped prior to use in the experiment. Urea fertilizer consisting of urea was prepared from livestock farms near the test site. Azolla was just harvested after rice was harvested from rice fields.

A tank (3 m³ in size) was used to produce vermicompost, which contained a population of active and stable earthworms (Eisenia andrei). Fertilizer application was performed on a reactor containing active earthworms. A mixture of cattle manure and rice straw was poured into the reactor, which supported a population density of 250 g of earthworm’s kg⁻¹ in the top layers. The upper surface of the reactor was divided into four chambers,
Table 3 Chemical analysis of the cattle manure and its vermicomposts.

| Parameter* | M     | VM    | VRM   | VAM   | VRAM  |
|------------|-------|-------|-------|-------|-------|
| pH         | 8.10 ± 0.13 | 7.43 ± 0.06 | 7.60 ± 0.00 | 6.83 ± 0.03 | 7.7 ± 0.01 |
| EC         | 1.08 ± 0.01 | 1.26 ± 0.03 | 0.96 ± 0.01 | 1.44 ± 0.01 | 1.38 ± 0.04 |
| Ash content (g/kg) | 197 ± 10.3 | 441 ± 21.7 | 239 ± 7.09 | 311 ± 14.28 | 291 ± 8.20 |
| Total OC (g/kg) | 412 ± 16.9 | 316 ± 6.5 | 439 ± 16.9 | 293 ± 8.92 | 336 ± 9.03 |
| Total OM (%) | 74.6 ± 2.3 | 49 ± 4.3 | 87.6 ± 2.3 | 45.7 ± 3.40 | 46.6 ± 1.12 |
| Total N (g/kg) | 16.8 ± 0.51 | 25.3 ± 1.54 | 21.6 ± 0.51 | 32.4 ± 1.55 | 27.2 ± 2.03 |
| Total P (g/kg) | 9.4 ± 0.91 | 15.4 ± 0.84 | 12.7 ± 0.91 | 17.2 ± 0.76 | 11.82 ± 0.07 |
| Total K (g/kg) | 10.4 ± 1.02 | 13.7 ± 1.11 | 10.1 ± 1.02 | 14.8 ± 0.34 | 13.46 ± 0.73 |
| C:N        | 24.46 ± 2.3 | 11.3 ± 0.47 | 17.14 ± 1.06 | 9.31 ± 0.24 | 12.35 ± 1.34 |
| Cu (mg/kg) | 135.2 ± 7.92 | 164.8 ± 13.8 | 152.0 ± 17.06 | 169.7 ± 11.09 | 155 ± 7.66 |
| Fe (mg/kg) | 215.4 ± 5.8 | 416.6 ± 5.7 | 372.1 ± 21.3 | 542.3 ± 13.76 | 484 ± 9.23 |
| Mn (mg/kg) | 109.7 ± 11.4 | 248.7 ± 4.6 | 212.0 ± 6.9 | 307.6 ± 9.51 | 260 ± 8.59 |
| Zn (mg/kg) | 184 ± 9.83 | 369 ± 12.06 | 342 ± 5.43 | 289.7 ± 14.5 | 319 ± 14.25 |

Note.
*Dry matter basis. Data are presented as mean ± standard error (n = 3)

and 45 kg of cattle manure along with rice straw were placed in three layers on top of each other (15 kg per layer) in each chamber, which was processed by earthworms. The moisture content of cattle manure in the vermi-reactor was maintained in the range of 75 to 80% and the sample was harvested from the last layer (two months after tillage) of the reactor (Lazcano, Gómez-Brandón & Domínguez, 2008).

The Vermicomposting process caused a significant change in cattle manure. The vermicompost was much darker in color and well-formed, and after the earthworm’s activity was processed into a homogeneous compound. The vermicomposting process helps to significantly reduce the pollution of organic matter in the environment and soil. Table 3 presents the physical, chemical, and nutritional properties of cattle manure and vermicompost. Cattle manure and vermicomposts were applied to the specified treatments 20 days before transplanting and mixed thoroughly with the soil. In the control, according to the results of soil decomposition, the required elements of chemical fertilizers were provided.

Bacterial inoculant preparation and sowing conditions

*Azospirillum brasilense* (strain BBU168) and *Azetobacter chroococcum* (strain SUAA4) isolated from rice roots in the paddy soils have been developed by the Soil Microbiology Research Institute of Soil and Water Research. The bacteria were cultured in improved medium and purified to a final concentration of $10^8$ CFU ml$^{-1}$. In the present study, the cultivated rice cultivar was Hashemi, the growth period of which varies from 118 to 140 days from planting date to physiological maturity. In order to treat the plants with bacteria, the roots of rice seedlings (twenty-seven days old) were placed separately in solutions containing 1000 ml of suspension of bacteria and bacteria for 2 h. In addition, for plants without inoculation, the roots of rice seedlings were placed in saline solution. Immediately after applying the bacterial treatments, the seedlings were planted at 0.2 × 0.2
m (three seedlings per mound) on the eighth of May. Weeds were controlled manually during the growing period and no herbicides were used. During the rice-growing period, the floodplain water level of the plateaus was constantly adjusted to 10 cm.

**Soil bacterial account and microbial biomass-C**

The soil of the rice rhizosphere was sampled at the tillering stage. Rhizosphere soil samples were collected by inserting a cylindrical cylinder 10 cm in diameter with a sharp edge at the bottom.

Soil microbial biomass-C was measured by modified chloroform fumigation–extraction method with fumigation at atmospheric pressure (Witt et al., 2000). Soil samples, 35 g on an oven-dry basis (48 h at 105 °C) were weighed into 500-ml glass Schott bottles and were fumigated by adding 2 ml of ethanol-free chloroform directly onto the soil. Microbial biomass C was estimated by extracting the fumigated soil with 0.5 M K₂SO₄ and extractable C determined by modified dichromate digestion of soil extract (Vance, Brookes & Jenkinson, 1987).

The total number of bacteria was determined using plate cultures and direct counting of microbes (Kelly, Haggblom & Tate, 1999). 2.10-g samples from each plot were sprayed separately into 250-ml Erlenmeyer containing 95 ml of sterile distilled water. The Erlenmeyer was rotationally mixed for 5 min, then they were fixed for 15 s allowing coarse particles to be deposited. 5 experimental tubes containing 9 ml of sterile distilled water were prepared. 1 ml of soil suspension was taken and transferred to tube No. 1. Once again, 1 ml was taken from the first tube and transferred to the second tube. The next dilutions were also prepared in the same way. The soil dilution was done to a dilution of 10⁻⁶ g/ml. For bacterial counting, dilutions of 10⁻⁴, 10⁻⁴ and 10⁻⁴ g/ml were selected and cultured in a special culture medium of aerobic heterotrophic bacteria, namely, Nutrient Agar with a concentration of 16 g/L. To prevent the growth of fungi, 50 mg/l of Nystatin was added to the culture medium of bacteria. After 25 h of incubation at 25 °C, colony counting was done.

**Urease activity**

Urease (EC 3.5.1.5) activity was measured in growing stages of rice (5 days after transplantation (T1), 1/₄ maximum tillering stage (30 days after transplantation) (T2), 1/₄ panicle initiation stage (70 days after transplantation) (T3) and 1/₄ maturity (100 days after transplantation) (T4)), according to the method proposed by Pattnaik et al. (1999). 20 g of air-dried soil (passed through a 2-mm sieve) was mixed with urea (20 ml) to provide a final concentration of 2,000 µg/g soil, and the suspensions were incubated for 5 h. The amount of residual urea present in the soil suspension upon incubation was determined by the non-buffer method introduced by Zantua & Bremner (1977). Urease activity was expressed as milligrams of urea hydrolyzed per gram of dry soil per hour.

**Plant sampling**

In the dough stage, 8 randomly chosen plants were removed from each plot, and chlorophyll was determined by Arnons’ method (1949) in the flag leaves.
After maturity stage, plant height, panicle height, the No. tillers hill⁻¹ and the yield of the seed (taking into account 14% moisture) were determined from 2 square meters per plot. The moisture content of the grains was measured using digital grain moisture meter (Model GMK–303R5–Korea) and the following equation was used to calculate the grain yield per plot by considering the moisture content of 14%:

\[
\text{Grain yield} = \frac{(100 - \text{moisture content of the sample}) \times \text{fresh grain weight}}{86}
\]

To determine the biomass, plants 1 square meter from the center of each plot were randomly harvested and then dried and weighed separately in a paper bag at 45 °C for 48 h (reported as biological function). The harvest index was also calculated using the following equation:

\[
\text{Harvest Index} = \frac{\text{Grain yield}}{\text{Biological yield}}
\]

The concentration of nitrogen (N) in the grain and straw samples was obtained after digestion in acid in the digestion block by the Kjeldal method. The protein concentration in the grain and straw was also calculated from the product of the amount of nitrogen in the grain and straw at 6.25. The concentrations of P and K in the grain and straw after digestion in a 9:1 ratio (HNO₃:HClO₄) of di-acide mixture were estimated using standard methods described by AOAC (1970). P and K uptake and grain protein yield were obtained by multiplying the dry weight of the grain by their concentration.

It should be noted that all treatments applied to rice, operations performed as well as measured traits in the first year and in the second year are exactly the same.

**Statistical analysis**

All data were subjected to Analysis of Variance (ANOVA) according to the methods described for factorial trial based on randomized complete block design combined over the years using SAS software 9.3. When F test indicated statistical significance at \( P < 0.01 \) or \( P < 0.05 \), the Least Significant Difference (LSD) was used to separate the means.

**RESULTS**

**Urease enzyme activity**

Urease enzyme activity of soil increased up to stage T3 in most organic amendments in both years of the experiment, but then (in stage T4) its activity decreased, and under non-inoculation conditions (Figs. 2A and 2B), the urease activity trend lines of the control treatment collided with the urease activity trend lines of organic amendments while, such a collision was not seen under inoculation of *Azotobacter* (Figs. 2C and 2D) and *Azospirillum* (Figs. 2E and 2F). Therefore, the effectiveness of organic amendments on enzyme activity in rhizosphere of inoculated plants increased compared to non-inoculated plants. In both years, VAM showed the most urease activity and *Azotobacter* seemed to show more enzyme activity compared to *Azospirillum* in response to the application of organic amendments.

**Soil bacterial account and microbial biomass–C**

The interaction effect of bacteria × organic amendments × year on microbial carbon biomass was significant \( P < 0.01 \) (Table 4). The mean comparison showed that, in the
first year (2017), compared to control, organic amendments increased microbial carbon biomass (24–45%) in non-inoculated plants, plants inoculated with *Azotobacter* (42–78%) and *Azospirillum* (31–64%). While in the second year (2018), this increase was obtained as 29–41%, 68–82%, and 63–71%, respectively in non-inoculated plants, plants inoculated with *Azotobacter* and *Azospirillum* compared to control. In both years, the highest microbial
Table 4 ANOVA of the effect of organic amendment on soil bacteria count (SBC), microbial biomass-C (MBC), chlorophyll a (CA), chlorophyll b (CB), total chlorophyll (TC), No. of tiller hill−1 (NTH), seed yield (SY), biological yield (BY), protein yield (PY), P uptake (PU), and K uptake (KU) under inoculation with PGPRs.

| S.O.V | Mean square (MS) |
|-------|-----------------|
|       | SBC | MBC | CA | CB | TC | NTH | SY | BY | PY | PU | KU |
| Y     | 14934ns | 789ns | 3.4ns | 1.07ns | 0.65ns | 0.31ns | 1595781ns | 3771791ns | 5698ns | 35.9ns | 1108ns |
| Y(R)  | 12449ns | 160ns | 20.8ns | 0.24ns | 1.07ns | 0.31ns | 474140ns | 790ns | 50.6ns | 93.9ns |
| B     | 81900ns | 3771791ns | 5698ns | 35.9ns | 1108ns | 424187ns | 327126ns | 9679459ns | 236372ns | 818ns |
| M     | 30723ns | 3771791ns | 5698ns | 35.9ns | 1108ns | 424187ns | 327126ns | 9679459ns | 236372ns | 818ns |
| Y × B | 1892ns | 3324497ns | 25543ns | 287ns | 484ns | 348570ns | 926332ns | 20414ns | 114ns | 265ns |
| Y × M | 5232ns | 3324497ns | 25543ns | 287ns | 484ns | 348570ns | 926332ns | 20414ns | 114ns | 265ns |
| B × M | 8293ns | 910690ns | 327126ns | 9679459ns | 236372ns | 818ns |
| Y × B × M | 8293ns | 910690ns | 327126ns | 9679459ns | 236372ns | 818ns |
| Error | 4975  | 9956  | 61.8  | 134  | 61.8  | 134  | 61.8  | 134  | 61.8  | 134  | 61.8  | 134  |

Note.
ns, not significant.
*significant at the 0.05 and 0.01 probability levels, respectively. S.O.V: source of variations, Y: year, R: replication, B: bacteria, M: organic amendments. The Y, B, and M are experimental factors whose main (individual) and interaction effects on the measured traits are included in this table.

**significant at the 0.05 and 0.01 probability levels, respectively. S.O.V: source of variations, Y: year, R: replication, B: bacteria, M: organic amendments. The Y, B, and M are experimental factors whose main (individual) and interaction effects on the measured traits are included in this table.

bacterial biomass-C was obtained from VAM under Azotobacter inoculation, although it did not statistically different with some treatments (Fig. 3A and 3B).

The number of bacteria was influenced by interaction effect of bacteria × organic amendments (P < 0.01) (Table 4). The mean comparison showed that, organic amendments increased the number of bacteria in non-inoculated plants by 22–32%, and it increased the number of bacteria by 22–32% both in plants inoculated with Azotobacter and Azospirillum. Although, there was no significant difference between VAM and some organic treatments in non-inoculated plants, but in the plants inoculated with Azotobacter and Azospirillum, the highest number of bacteria was obtained from the VAM (Fig. 4).

Chlorophyll content
Chlorophyll content of flag leaf was influenced by interaction effect of organic amendments × bacteria (Table 4). The plants treated by N₂-fixing bacteria increased the effect of organic amendments on chlorophyll content, so that the content range of chlorophyll a was obtained as 4.5–1.9, and 7.3–4.3 mg/L, respectively (Fig. 5) in non-inoculated plants and inoculated plants, and the content range of chlorophyll b was obtained as 1.0–0.4, and 0.7–2.8 mg/L, respectively (Fig. 6) in non-inoculated and inoculated plants. Under non-inoculation conditions, although organic amendments of total chlorophylls (chlorophyll a + b) increased by 16–52% compared to control, but this increase was obtained as 23–79% and 11–67%, respectively (except VM) under Azotobacter and Azospirillum inoculation conditions. N₂-fixing bacteria played a positive role in improving flag leaf chlorophyll content especially in vermicomposts containing Azolla. The highest content of total chlorophyll was obtained from VRAM, VRM, and VAM under Azotobacter inoculation, and from VRAM under Azospirillum inoculation (Fig. 7).
Figure 3  Mean comparison of effect of organic amendments on soil microbial biomass–C under inoculation with N<sub>2</sub>-fixing bacteria in 2017 (A) and 2018 (B).

Full-size DOI: 10.7717/peerj.10833/fig-3

Figure 4  Mean comparison of effect of organic amendment on viable anaerobic bacteria count ($\times 10^5$ CFU ml<sup>−1</sup>) under inoculation with N<sub>2</sub>-fixing bacteria.

Full-size DOI: 10.7717/peerj.10833/fig-4

**Tiller number, grain yield, biological yield and HI**

Tiller number, grain yield and biological yield were influenced by interaction effect of bacteria × organic amendments × year but HI was influenced by interaction effect of organic amendments × bacteria (Table 4). In both years, organic amendments significantly increased the number of tillers compared to control and in inoculated plants, there were more tillers, so that tillers of organic amendments were obtained as 16–45% under
non-inoculation conditions in the first year compared to control and in the second year they were obtained as 19–53%. Under the non-inoculation condition with *Azotobacter* in the first year, they increased by 34–75% and in the second year, they increased by 51–86%. Under the non-inoculation condition with *Azospirillum* in the first year they increased by 29–73% and in the second year, they increased by 19–78%. In both years, the maximum number of tiller was obtained from VAM and VARM under inoculation with *Azotobacter* (Table 5).
Maximum grain yield was obtained from vermicompost treatments when plants were inoculated with N₂-fixing bacteria. Meanwhile, grain yield of control increased by 9.7 and 6.4%, respectively in the first year under inoculation with Azotobacter and Azospirillum, and in the second year under inoculation with Azotobacter and Azospirillum, the grain yield increased by 19 and 15%, respectively. But the grain yield increased by 29–56% in the first year and 41–83% in the second year with application of organic amendments under inoculation with Azotobacter, and under inoculation with Azospirillum it increased by 20–43% in the first year and by 33–74% in the second year.

It should be noted that, the application of manure without vermicomposting (M) increased grain yield by 6, 29, and 20% in the first year, and 9.4, 41, and 33% in the second year, respectively under non-inoculation, inoculation with Azotobacter and Azospirillum compared to the control, while vermicomposting manure (VM) increased grain yield by 15, 37, and 31% in the first year, and 48, 73, and 64% in the second year, respectively under non-inoculation, inoculation with Azotobacter and Azospirillum compared to the control. In both years, the maximum grain yield was obtained from VAM + Azotobacter (Table 5).

The biological yield of the plants changed in response to organic amendments × bacteria, so that the application of organic amendments increased biological yield by 22–58% in the first year and by 28–45% in the second year under non-inoculation conditions, it increased by 44–86% and 31–66%, respectively in the first and second years under the inoculation with Azotobacter compared to the control, and increased by 33–50% and 28–52%, respectively in the first and second years under the inoculation with Azospirillum compared to the control. Among all organic amendments, VAM (vermicompost of manure + Azolla) showed the highest biological yield under both non-inoculation and inoculation conditions (Table 5).
Table 5  Mean comparison of the effect of organic amendments on plant height, panicle height, no. tillers hill\(^{-1}\), seed yield, biological yield and harvest index under inoculation with N\(_2\)-fixing bacteria.

| Bacteria       | Organic | Plant height (cm) | Panicle height (cm) | No. of tiller hill\(^{-1}\) | Grain yield (kg/hm\(^2\)) | Protein yield (kg/hm\(^2\)) | Biological yield (kg/hm\(^2\)) |
|----------------|---------|-------------------|---------------------|-----------------------------|-----------------------------|-------------------------------|-------------------------------|
|                |         |                   |                     |                             |                             |                               |                               |
|                | 2017    |                   |                     |                             |                             |                               |                               |
| Control        | 110.1e  | 25.60e            | 12.3i               | 2690g                       | 274h                        | 5814j                         |
| M              | 124.3d  | 29.66c–e          | 15.5i               | 2857fg                      | 341e                        | 7095hi                        |
| VM             | 128.9b–d| 28.27c–e          | 18.66c–g            | 3095c–g                     | 332f                        | 7161hi                        |
| Non-inoculum   |         |                   |                     |                             |                             |                               |                               |
| VRM            | 126.2cd | 29.62c–e          | 18.0d–h             | 2881fg                      | 291gh                       | 7124hi                        |
| VAM            | 131.0b–d| 36.56abc          | 19.3b–e             | 3595b–f                     | 318f                        | 8757d                         |
| VRAM           | 126.3cd | 34.90–d           | 19.0c–f             | 3500b–f                     | 381d                        | 7490gh                        |
| Control        | 124.2d  | 29.63c–e          | 16.3h               | 2985d–g                     | 278gh                       | 7833e–h                       |
| M              | 131.7b–d| 30.63c–e          | 16.66gh             | 3476b–g                     | 331f                        | 8381d–f                       |
| Azetobacter    |         |                   |                     |                             |                             |                               |                               |
| VM             | 134.8a–c| 33.00b–e          | 20ab–d              | 3690b–e                     | 334ef                       | 9890bc                        |
| VAM            | 132.0b–d| 28.85c–e          | 18.0d–h             | 3428b–g                     | 338e                        | 9047cd                        |
| VRAM           | 134.5a  | 43.22a            | 22.6a               | 4667a                       | 438a                        | 10833a                        |
| Control        | 137.9ab | 40.37ab           | 21.6ab              | 4214ab                      | 366d                        | 10057ab                       |
| Azospirillum   |         |                   |                     |                             |                             |                               |                               |
| M              | 126.7cd | 29.52c–e          | 17.0f–h             | 3238c–g                     | 333ef                       | 8347d–g                       |
| VM             | 130.3b–d| 34.01b–d          | 17.667e–h           | 3547b–f                     | 347e                        | 7771e–h                       |
| VAM            | 129.4b–d| 35.61a–d          | 18.0d–h             | 3295c–g                     | 371d                        | 6505ij                        |
| VRAM           | 135.6a–c| 39.06ab           | 21.3ab              | 3857bc                      | 424b                        | 9209b–d                       |
| 2018           |         |                   |                     |                             |                             |                               |                               |
| Control        | 176.6i  | 27.10d            | 11.6h               | 2309j                       | 249i                        | 6309i                         |
| M              | 126.0f–h| 28.58cd           | 16.33g              | 2928g–j                     | 190j                        | 8095gh                        |
| VM             | 126.7f–h| 35.13a–d          | 18def               | 3428e–h                     | 242i                        | 8566d–g                       |
| Non-inoculum   |         |                   |                     |                             |                             |                               |                               |
| VRM            | 132.6f–h| 30.41cd           | 17.01ef             | 3214f–i                     | 283h                        | 8467e–h                       |
| VAM            | 126.0f–h| 33.87a–d          | 18.0d–f             | 3942b–d                     | 340d–f                      | 9200b–d                       |
| VRAM           | 131.3d–g| 40.13ab           | 17.33ef             | 3666c–f                     | 280h                        | 8809d–f                       |
| Control        | 124.5g–i| 27.26d            | 13.5gh              | 2761h–j                     | 320f                        | 7847h                         |
| Azetobacter    |         |                   |                     |                             |                             |                               |                               |
| VM             | 131.7d–f| 31.54bc           | 19.33e–f            | 4100bc                      | 296gh                       | 9680a–c                       |
| VAM            | 150.2a  | 40.44a            | 22.33a              | 5081a                       | 404a                        | 10323a                        |
| VRAM           | 142.2bc | 36.74a–c          | 21.33a–c            | 4452ab                      | 352c–e                      | 9753ab                        |
| Control        | 120.1hi | 27.85d            | 12.5h               | 2667ij                      | 290h                        | 6095i                         |
| Azospirillum   |         |                   |                     |                             |                             |                               |                               |
| M              | 127.6e–g| 29.26cd           | 17.0ef              | 3500d–g                     | 303gh                       | 8123f–h                       |
| VM             | 130.3d–g| 34.45a–d          | 18.66c–f            | 3857b–f                     | 367bc                       | 8795d–f                       |
| VAM            | 134.1d–e| 34.68a–d          | 18def               | 3782b–f                     | 364b–d                      | 8605d–g                       |
| VRAM           | 135.6cd | 36.59a–d          | 21.66ab             | 4357bc                      | 379b                        | 9623bc                        |

**Protein yield**

The interaction effect of organic amendments × bacteria × year on protein yield was significant \((P < 0.01)\) (Table 4). In both years, organic amendments had significant
superiority compared to the control. In both years, under non-inoculation and inoculation with *Azotobacter* the highest protein yield was obtained from plants treated with VAM, but under inoculation conditions with *Azospirillum*, there was no significant difference between VAM and VRAM. Among all treatments, in both years, the highest protein yield was obtained from *Azotobacter* + VAM (438 kg/hm$^2$ in the first year and 404 kg/hm$^2$ in the second year). It is worth noting that, although grain yield of treatments was less in the first year compared to the second year, but the protein yield was higher in the first year (Table 5).

**P and K uptake**

K and P uptake by grain were influenced by interaction effect of organic amendments × bacteria (Table 4). The mean comparison of interaction effect of organic amendments × bacteria on P uptake showed highest P uptake was obtained under non-inoculation conditions by VAM and VARM (with a 76 and 79% increase compared to the control), under inoculation conditions with *Azotobacter* by VAM and VM (with a 44 and 35% increase compared to the control + *Azotobacter*), and also under inoculation conditions with *Azospirillum* by VAM (with a 63% increase compared to the control + *Azospirillum*) (Fig. 8).

The mean comparison of the interaction effect of organic amendments × bacteria on K uptake showed highest K uptake under non-inoculation conditions, and under inoculation with *Azospirillum* by VAM (with a 51% increase compared to control), and VRAM and VAM (with a 80 and 74% increase compared to control + *Azospirillum*). But in plants
inoculated with *Azotobacter*, there was no significant difference between vermicompost treatments including VM, VM, VAM, and VARM, but significantly increased K uptake compared to the manure treatment (M) and control (Fig. 9). Also, control + *Azotobacter* increased K and P uptake by 25 and 32%, respectively, and control + *Azospirillum* increased K and P uptake by 8.8 and 19%, respectively compared to control + non-inoculation.

**DISCUSSION**

The results showed that, vermicomposting of manure with and without *Azolla* and rice straw and their application as organic fertilizers under inoculation with N\textsubscript{2}-fixing bacteria, especially *Azotobacter*, and increased the number and biomass-C of soil microorganisms and urease enzyme activity. The number and biomass-C of soil microorganisms and urease enzyme activity were lowest in control plots due to high stress and inadequate nutrient supply, lack of using organic fertilizer and lower amounts of rhizodeposition (root exudates and root biomass). These results are consistent with the findings of the studies by *Cooper & Warman* (1997); *Bhattacharyya, Chakrabarti & Chakraborty* (2005); *Kopecky et al.* (2011); and *Tu et al.* (2017). An increase in the number and biomass-C of microorganisms in planted rice under flooded condition has been reported following the application of vermicompost (*Nayak, Babu & Adhya, 2007*). *Albiach et al.* (2000) and *Cui et al.* (2018) reported that, annual application of adequate amounts of some organic residues led to significant increase in soil enzyme activities.

The inoculation of plants with *Azotobacter* and *Azospirillum* increased the application efficiency of organic fertilizers in improving the number and biomass-C of soil microorganisms and urease enzyme activity. The positive role of N\textsubscript{2}-fixing bacteria in increasing the population of Diazotrophic bacteria, improving enzyme activity and microbial biomass N in rice rhizosphere has been confirmed in the studies by *Kumar,*
Swain & Bhadoria (2018) and Zhang et al. (2018). Mahanta et al. (2012) reported that, vermicomposting of manure increased the soil microbial activity by 79% and biomass-C by 68% compared to manure, resulting from improvements in soil physical and chemical activity by vermicompost.

In both years of experiment and in all treatments, the urease activity increased to stage T3 (\(\frac{1}{4}\) panicle initiation stage), due to increased microbial activity of the soil, but decreased in the stage T4 (\(\frac{1}{4}\) maturity). Although, the organic treatments increased the urease activity, but inoculation of plants with Azotobacter and Azospirillum increased the effect of organic amendments on urease activity and among the treatments, VAM + Azotobacter showed the most urease activity. In agreement with our results, Rodrigues, Ladeira & Arrobas (2018) reported that, the organic material containing Azotobacter increased the bioavailability of N over organ, by an additional N\(_2\)-fixing value of 11.4 kg/hm\(^2\) estimated from the six crops of the field experiment. Nayak, Babu & Adhya (2007) reported increased urease activity with a compost made of residues of rice + urea in flooded rice, and Arora & Kaur (2019) reported about Aspergillus terreus-enriched organic manures. The increase of total organic carbon, water-soluble carbohydrate, water-soluble C, and proliferation and microbial activity of the soil are among the most important contributions of organic amendments (Srivastava, Aragno & Sharma, 2010) resulting in the release of elements in the soil and organic materials and making available roots by increasing the bacterial activity (Pérez-Piqueres et al., 2006). In fact, changes in the composition of microbial communities have been observed as a result of incorporating inorganic or organic amendments (Marschner, Kandeler & Marschner, 2003; Crecchio et al., 2004). But various properties of organic materials can have a different effect on soil microbiota and will strongly influence the microbial use of the C contained in these materials. Vermicomposting increased the number and biomass-C of microorganisms of the soil, especially under inoculation with Azotobacter, compared to the manure treatment (M) and control. Carbohydrates would act as sources of energy in respiration of microbiota (Zhong et al., 2010; Li et al., 2015) showing intense temporal changes due to their continuous process of synthesis and degradation. This is reflected by the changes in urease activity during the growing season of rice under various treatments. During the growing season, urease activity was higher in treatments under inoculation compared to non-inoculation treatments, especially VAM and VRAM treatments that showed more enzyme activity under inoculation with Azotobacter compared to Azospirillum. Higher urease activity under the application of VAM and VRAM treatments demonstrated higher N-fixing ability of Azotobacter, which increased the available N content in the rice rhizosphere.

Zhang et al. (2017) and Islam et al. (2012) also suggested that, wide variety of free-living N\(_2\)-fixing bacteria can be used as a feasible alternative to N fertilizer in rice ecosystems. The addition of organic material to soil results in an increase in the total organic carbon content increasing the proliferation and activity of microorganisms (Martinez-Balmori et al., 2013), therefore in the beginning of the growth, although the enzyme activity of microorganisms is high, but they take out the nutrients from the plant and spend them for their bodies (Rodrigues, Ladeira & Arrobas, 2018).
Therefore, the reason for the superiority of Azolla-containing organic treatments, namely VAM (vermicompost of manure + Azolla) and VRAM (vermicompost of manure + Azolla + rice straw), can be due to more amounts of N compared to other treatments. The application of Azolla compost in paddy rice has been studied in many countries and its positive effect in increasing the crop has been well proved (Razavipour et al., 2018). The preferred effect of VAM and VRAM on the microbial population of the soil and the urease activity, as well as the growth and yield of rice in inoculated plants is evident. In this regard, Zhang et al. (2018) reported that, inoculation of A. brasilense and P. fluorescens in the rice rhizosphere accelerated N transformations and improved the N-supplying capacity of the rhizosphere soil, and increased rice biomass. The most beneficial effects were observed with A. brasilense and P. fluorescens co-inoculation in the rice rhizosphere. Inoculation of microorganisms is influenced by agricultural practices (Velusamy, Immanuel & Gnanamanickam, 2013) improving both growth and nutrient acquisition (Khan, 2018) of rice grown under upland conditions (Rajeshkannan, Sumathi & Manian, 2009; Zhang et al., 2017).

The used treatments have improved the biological, physical, and chemical properties of the soil and increased the growth of morphological traits. So that in both years, under both non-inoculation and inoculation conditions, application of organic amendments compared to control increased plant and panicle height. The highest plant and panicle height was obtained from plants treated with VAM and VRAM under inoculation with Azotobacter. Kumar, Swain & Bhadoria (2018) and Lin, Zhu & Lin (2011) reported that, the number of functional leaves, leaf area and total number of tillers was higher in plants treated with organic nutrient, which increased the photosynthetic rate leading to higher plant height.

Moreover, the increase in photosynthesis of the plant depends on the content of leaf chlorophyll (Murchie et al., 2002; Singh, Singh & Sharma, 2013) as influenced by organic amendments and bacterial inoculation in this experiment, and the highest chlorophyll of a, b and total chlorophyll was observed in vermicompost treatments under inoculation with Azotobacter. In most studies, the increase in chlorophyll content of leaf has been reported to be directly associated with the absorption of N and Fe (Hosseinzadeh, Amiri & Ismaili, 2018). In addition to atmospheric N fixation, the solubility of minerals such as P, K, Cu, and Fe and the production of sidrophor are among the important effects of Azotobacter and Azospirillum (Yadav et al., 2014; Rodrigues, Ladeira & Arrobas, 2018). On the other hand, vermicompost is also rich in some macro and micro elements (Garcia et al., 2014; Yadav et al., 2014; Eo & Park, 2019). Therefore, vermicompost involves desirable properties such as a high capacity for cation exchange, increased absorption of nutrients and other beneficial physical, biological, and chemical properties (Ludibeth, Marina & Vicenta, 2012) increasing the chlorophyll content and stability of the photosynthetic system of rice plants. The vermicompost application led to a decrease in the Reactive Oxygen Species (ROS) production, increased accessibility to nutrients and the required elements for biochemical activity (Baghbani-Arani & Modarres-Sanavy, 2017). Mahanta et al. (2012) and Shirkhani & Nasrolahzadeh (2016) reported increased chlorophyll content of leaves by organic fertilizers and N2-fixing bacteria. Increasing the leaf chlorophyll content
leads to an increase in photosynthetic capacity. The acceleration of photosynthesis leads to an increase in plant height, number of tillers and number of grain per panicle (Yi-hu et al., 2014). Garcia et al. (2016) reported that, vermicompost increased the number of tillers and plant dry weight by producing humic acid and supplying nutrients, especially N and K.

Meena & Shivay (2010) introduced the improvement of rhizosphere in increasing the root activity as a factor increasing the number of tillers. In our experiment, in both years, bacterial treatments × organic amendments had a positive effect on the number of tillers. Although the number of tillers is dependent on environmental and genetic factors, but environmental conditions, especially nutrition have highest effect in the early stages of growth (Murchie et al., 2002; Nuensi et al., 2018). In both years, vermicomposts containing Azolla under inoculation with Azotobacter produced greatest tillers. Previous studies revealed that, these management practices have amended the soil structure and function, as well as nutrient availability influencing the plant height, number of tiller, panicle length, biological and grain yield of rice by the synergistic effects (Simarmata et al., 2016; Tsujimoto et al., 2009; Thakur et al., 2010; Kumar, Swain & Bhadoria, 2018). The combined use of vermicompost + N2-fixing bacteria is better than sole application of them for grain, straw and biological yields, because organic fertilizers can reduce N loss (Kumar & Singh, 2001) and maintain the supply of N to rice plants for a longer time (Nayak, Babu & Adhya, 2007; Li et al., 2015).

In the present experiment, in both years, the grain and biological yield of inoculated plants increased significantly by applying different vermicomposts compared to non-inoculated plants under similar treatments. The highest grain yield by VAM + Azotobacter treatment in the first and second years was obtained as 4,667 and 5,081 kg/hm², respectively, although there was no statistically significant difference in some of the treatments. In both inoculated and non-inoculated plants, VAM treatment produced the highest biological yield and VRAM treatment ranked the next, in addition, the maximum biological yield was observed under inoculation with Azotobacter. Eo & Park (2019) also stated the role of vermicompost in increasing rice growth and yield, so that the vermicompost application has altered the resource allocation and soil chemical properties leading to establishment of new interactions between root parameters and components. In most studies similar to our study, the main cause of increase in the growth and grain yield of rice by applying organic fertilizers under inoculation with N2-fixing bacteria has been introduced due to the increase in the availability and supply of N in different stages of rice growth. In our experiment, due to the application of bacteria × vermicompost, the amount of fixed atmospheric N2 and organic materials content was enhanced in the soil.

Marginal increase in N content of rice straw due to inoculation with Azotobacter and use of organic fertilizers has been reported (Rodrigues, Ladeira & Arrobas, 2018), resulting from an increase in the N availability through synchronized released from the inoculation of N2-fixing bacteria, which increased the N concentration proportionately in grains and straw and finally led to higher N uptake with the highest amount of N (Li et al., 2015; Tu et al., 2017). In our study, in both years, protein yield significantly increased in plots treated with both vermicompost under inoculation and non-inoculation conditions compared to control and M treatments. In non-inoculated and inoculated plants with Azotobacter,
the highest protein yield was obtained from VAM, but there was no significant difference between VAM and VRAM in plants inoculated with *Azospirillum*. Tejada & González (2009), Bejbaruah, Sharma & Banik (2013), Mengi et al. (2016) and Taheri Rahimabadi, Ansari & Razavi Nematollahi (2018) have reported the effect of vermicompost and organic amendments on increasing the grain protein of rice. The higher rice yield using the integrated N management might be due to the increase in number of filled grains per panicle, the number of panicles per plant, and 1,000-grain weight (Amanullah, 2016). The difference in nutrient uptake from different N source combinations significantly influences growth and yield potential (Gu et al., 2014). Myint et al. (2010) reported that, organic amendments can increase yield through the improvement of soil water holding capacity, physical and chemical conditions, reducing volatilization of nitrogenous fertilizers to NH₃ gas and the greater availability of plant nutrients for a longer time.

The P and K uptake of grain were influenced by interaction effect of organic amendments × bacteria. The highest P uptake in non-inoculated plants was obtained from VAM and VARM (with a 76 and 79% increase compared to control). In plants inoculated with *Azotobacter*, VAM and VM treatments showed the highest P uptake, but in plants inoculated with *Azospirillum*, VAM treatment showed the highest P uptake. The highest K uptake in non-inoculated plants was obtained from VAM treatment and it was obtained in plants inoculated with *Azospirillum* from VRAM and VAM. But in plants inoculated with *Azotobacter*, there was no significant difference between vermicompost treatments including VM, VRM, VAM, and VARM, but significantly increased K uptake compared to the manure (M) and control. Our results confirm the findings of the study by Yadav et al. (2014) who have reported 50% increase in content and uptake of N, P, and K in wheat using PGPR strains. An increase in P uptake over control may be due to the bacterial solubilization of insoluble phosphate in soil. These bacteria showed an effective role in P uptake and growth promotion of plants by dissolution of inorganic insoluble phosphate as reported in the study by Narula et al. (2000).

These results showed that, the application of organic amendments + growth stimulating bacteria leads to an increase in organic material pools and nutrient availability, as well as amendment of the physical environment of soil and rice yield (Chen 2017). Additionally, the application of vermicompost, especially VAM and VARM treatments, either alone or together with *Azotobacter* and *Azospirillum*, in addition to increasing the number and biomass-C of soil microorganisms, leads to improvement of the solubility of nutrients, especially N, P, K and some microelements.

In line with our results, Zhong et al. (2010), Li et al. (2015); Wang et al. (2015) and Cui et al. (2018) reported that, the application of organic fertilizers influences on soil physicochemical and biological properties, especially regarding soil pH and microbial biomass C. Nayak, Babu & Adhya (2007) showed that, soil urease activity had a significant negative relationship with Eh and had a positive relationship with the content of N, P, and Fe. Therefore, application of vermicompost and N₂-fixing bacteria by changing the state of redox and soil pH and catalytic efficiency may influence the accumulation and activity of enzymes, and importantly increases the availability of nutrients (Wlodarzyk, Stepniewski & Brzezinska, 2002). In this regard, Basha, Basavarajappa & Hebsur (2017)
and Taheri Rahimabadi, Ansari & Razavi Nematollahi (2018) reported the effect of vermicompost application on increasing the biomass and grain yield of rice due to the increased absorption of essential elements of plant growth. Similarly, Yadav et al. (2014) reported that, Azotobacter and Azospirillum inoculated with rice seedling led to a 35–21% increase in the yield and yield components, respectively compared to control under field condition. There were significant interactions between nitrogen level and biofertilizer regarding yield, yield component, and protein content (Nosheen et al., 2016). In this study, Azotobacter and Azospirillum also acted complementarily regarding positive effects of vermicomposts on nutrients absorption and rice yield.

CONCLUSION
The results of the present study showed that, vermicomposting of cow manure, rice straw and Azolla led to improvement of physicochemical properties of used materials. In both years, the application of vermicomposts had a positive effect on the number and biomass-C of soil microorganisms and urease enzyme activity. It was found that, these changes led to the amendment of physical and biological properties of the soil and increased the chlorophyll content, biological and grain yield, and the uptake of N, P, and K by the availability of nutrients. In this study, vermicomposts containing Azolla (VAM and VARM) were more beneficial for increasing the rice productivity. Inoculation of the plants with Azotobacter and Azospirillum also showed significant superiority in the above mentioned traits compared to non-inoculated plants. On the other hand, the synergistic effect of bacteria with organic amendments, especially with Azotobacter was observed with vermicomposts containing Azolla, so that the highest grain yield in the first and second years with an average of 4667 and 5081 kg/hm², respectively was obtained from VAM + Azotobacter. The present study provides information on the effect of applying manure and various vermicomposts on the number and biomass-C of soil microorganisms and urease enzyme activity and finally on the absorption of elements and grain yield under inoculation with N₂-fixing. Our results suggested that, applying organic source for vermicomposting has a great effect on the enzymatic and biological activity of the soil under flooded conditions contributing to the sustainable development of agro-ecosystems. It is suggested to evaluate the vermicomposting effect on various organic sources weak and rich in nutrients, especially N, along with inoculation of plants or vermicompost enrichment with N₂-fixing bacteria in future studies.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding
This research was partially supported by a research grant (UOZ-GR-9618-25) provided by University of Zabol. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures
The following grant information was disclosed by the authors:
Competing Interests
The authors declare there are no competing interests.

Author Contributions
• Mehdi Ghadimi conceived and designed the experiments, performed the experiments, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
• Alireza Sirousmehr and Ahmad Ghanbari conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
• Mohammad Hossein Ansari conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

Data Availability
The following information was supplied regarding data availability:
Raw data are available in a Supplemental File.

Supplemental Information
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.10833#supplemental-information.

REFERENCES
Albiach R, Canet R, Pomares F, Ingelmo F. 2000. Microbial biomass content and enzymatic activities after the application of organic amendments to a horticultural soil. Bioresource Technology 75(1):43–48 DOI 10.1016/S0960-8524(00)00030-4.
Amanullah H. 2016. Influence of organic and inorganic nitrogen on grain yield and yield components of hybrid rice in Northwestern Pakistan. Rice Science 23(6):326–333 DOI 10.1016/j.rsci.2016.02.007.
Arnon DI. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiology 24(1):1–15 DOI 10.1104/pp.24.1.1.
Arora M, Kaur A. 2019. Scanning electron microscopy for analysing maturity of compost/vermicompost from crop residue spiked with cattle dung, Azolla pinnata and Aspergillus terreus. Environmental Science and Pollution Research 26(2):1761–1769 DOI 10.1007/s11356-018-3673-8.
Ashoori D, Allahyari MS, Damalas CA, Bagheri A. 2018. Challenges for efficient land use in rice production of northern Iran: the use of modern cultivars among small—scale farmers. Land Use Policy 76:29–35 DOI 10.1016/j.landusepol.2018.04.044.
Baghbani-Arani A, Modarres-Sanavy SAM. 2017. Towards improving the agronomic performance, chlorophyll fluorescence parameters and pigments in fenugreek using zeolite and vermicompost under deficit water stress. Industrial Crops and Products 109:346–357 DOI 10.1016/j.indcrop.2017.08.049.
Basha SJ, Basavarajappa R, Hebsur NS. 2017. Nutrient uptake as influenced by organic and inorganic sources of nutrients under aerobic rice cultivation. *Environment and Ecology* **35**(1B):474–479.

Bejbaruah R, Sharma RC, Banik P. 2013. Split application of vermicompost to rice (*Oryza sativa* L.): its effect on productivity, yield components, and N dynamics. *Organic Agriculture* **3**(2):123–128 DOI 10.1007/s13165-013-0049-8.

Bhattacharjee RB, Singh A, Mukhopadhyay SN. 2008. Use of nitrogen-fixing bacteria as biofertiliser for non-legumes: prospects and challenges. *Applied Microbiology and Biotechnology* **80**:199–209 DOI 10.1007/s00253-008-1567-2.

Bhattacharyya P, Chakrabarti K, Chakraborty A. 2005. Microbial biomass and enzyme activities in submerged rice soil amended with municipal solid waste compost and decomposed cow manure. *Chemosphere* **60**:310–318 DOI 10.1016/j.chemosphere.2004.11.097.

Campitelli P, Velasco M, Ceppi S. 2012. Characterization of humic acids derived from rabbit manure treated by composting-vermicomposting process. *Journal of Soil Science and Plant Nutrition* **12**(4):875–891.

Chen X, Liu M, Kuzyakov Y, Li W, Liu J, Jiang C, Li Z. 2018. Incorporation of rice straw carbon into dissolved organic matter and microbial biomass along a 100-year paddy soil chronosequence. *Applied Soil Ecology* **130**:84–90 DOI 10.1016/j.apsoil.2018.06.004.

Chen D, Yuan L, Liu Y, Ji J, Hou H. 2017. Long-term application of manures plus chemical fertilizers sustained high rice yield and improved soil chemical and bacterial properties. *European Journal of Agronomy* **90**:34–42 DOI 10.1016/j.eja.2017.07.007.

Cooper JM, Warman PR. 1997. Effects of three fertility amendments on soil dehydrogenase activity, organic C and pH. *Canadian Journal of Soil Science* **77**:281–283 DOI 10.4141/S96-023.

Crecchio C, Curci M, Pizzigallo MD, Ricciuti P, Ruggiero xx. 2004. Effects of municipal solid waste compost amendments on soil enzyme activities and bacterial genetic diversity. *Soil Biology and Biochemistry* **36**(10):1595–1605 DOI 10.1016/j.soilbio.2004.07.016.

Cui X, Zhang Y, Gao Jusheng, Gao FPP. 2018. Long—term combined application of manure and chemical fertilizer sustained higher nutrient status and rhizospheric bacterial diversity in reddish paddy soil of Central South China. *Nature* **8**:16554.

El-Haddad ME, Zayed MS, El-Sayed GAM, Hassanein MK, El-Satar AA. 2014. Evaluation of compost, vermicompost and their teas produced from rice straw as affected by addition of different supplements. *Annals of Agricultural Sciences* **59**(2):243–251 DOI 10.1016/j.aoas.2014.11.013.

Eo J, Park KC. 2019. Effect of vermicompost application on root growth and ginsenoside content of Panax ginseng. *Journal of Environmental Management* **234**:458–463.

Garcia AC, Santos LA, De Souza LGA, Tavares OCH, Zonta E, Gomes ETM, Mina JMG, Berbara RLL. 2016. Vermicompost humic acids modulate the accumulation and metabolism of ROS in rice plants. *Journal of Plant Physiology/TD* **192**:56–63 DOI 10.1016/j.jplph.2016.01.008.
Garcia AC, Santos LA, Izquierdo FG, Rumjanek VM, Castro RN, Dos Santos FS, De Souza LGA, Berbara RLL. 2014. Potentialities of vermicompost humic acids to alleviate water stress in rice plants (Oryza sativa L.). *Journal of Geochemical Exploration* **136**:48–54 DOI 10.1016/j.gexplo.2013.10.005.

Gu UD, Liu LJ, Wang ZQ, Zhang H, Yang JC. 2014. Changes in grain yield of rice and emission of greenhouse gases from paddy fields after application of organic fertilizers made from maize straw. *Rice Science* **21**(4):224–232 DOI 10.1016/S1672-6308(13)60187-0.

Guridi I, Calderin G, Louro B, Martinez B, Rosquete B. 2017. The humic acids from vermicompost protect rice (Oryza sativa L.) plants against a posterior hidric stress. *Cultivos Tropicales* **38**(2):53–60.

Hosseinzadeh SR, Amiri H, Ismaili A. 2018. Evaluation of photosynthesis, physiological, and biochemical responses of chickpea (Cicer arietinum L. cv. Pirouz) under water deficit stress and use of vermicompost fertilizer. *Journal of Integrative Agriculture* **17**(11):2426–2437 DOI 10.1016/S2095-3119(17)61874-4.

Islam MR, Sultana T, Cho JC, Joe MM, Sa TM. 2012. Diversity of freeliving nitrogen-fixing bacteria associated with Korean paddy fields. *Annals of Microbiology* **62**:1643–1650 DOI 10.1007/s13213-012-0421-z.

Kelly JJ, Haggbloom M, Tate III RL. 1999. Changes in soil microbial communities over time resulting from one time application of zinc: a laboratory microcosm study. *Soil Biology and Biochemistry* **31**:1455–1465 DOI 10.1016/S0038-0717(99)00059-0.

Khan HI. 2018. Appraisal of biofertilizers in rice: to supplement inorganic chemical fertilizer. *Rice Science* **25**(6):357–362 DOI 10.1016/j.rsci.2018.10.006.

Kopecky J, Kyselkova M, Omelka M, Cermak L, Novotna J, Grundmann GL, Loccoz Y, Sagova-Mareckova M. 2011. Actinobacterial community dominated by a distinct clade in acidic soil of a waterlogged deciduous forest. *FEMS Microbiology Ecology* **78**(2):386–394 DOI 10.1111/j.1574-6941.2011.01173.x.

Kumar V, Singh KP. 2001. Enriching vermicompost by nitrogen fixing and phosphate solubilizing bacteria. *Bioresource Technology* **76**(2):173–175 DOI 10.1016/S0960-8524(00)00061-4.

Kumar KA, Swain DK, Bhadoria PBS. 2018. Split application of organic nutrient improved productivity, nutritional quality and economics of rice-chickpea cropping system in lateritic soil. *Field Crops Research* **223**:125–136 DOI 10.1016/j.fcr.2018.04.007.

Ladha JK, Reddy PM. 2003. Nitrogen fixation in rice systems: state of knowledge and future prospect. *Plant Soil* **252**:151–167 DOI 10.1023/A:1024175307238.

Lazcano C, Gómez-Brandón M, Domínguez J. 2008. Comparison of the effectiveness of composting and vermicomposting for the biological stabilization of cattle manure. *Chemosphere* **72**(7):1013–1019 DOI 10.1016/j.chemosphere.2008.04.016.

Li J, Cooper JM, Lin ZA, Li Y, Yang X, Zhao B. 2015. Soil microbial community structure and function are significantly affected by long-term organic and mineral fertilization regimes in the North China Plain. *Applied Soil Ecology* **96**:75–87 DOI 10.1016/j.apsoil.2015.07.001.
Lim SL, Lee LH, Wu TY. 2016. Sustainability of using composting and vermicomposting technologies for organic solid waste biotransformation: recent overview, greenhouse gases emissions and economic analysis. *Journal of Cleaner Production* 111:262–278 DOI 10.1016/j.jclepro.2015.08.083.

Lim SL, Wu TY, Sim EYS, Lim PN, Clarke C. 2012. Biotransformation of rice husk into organic fertilizer through vermicomposting. *Ecological Engineering* 41:60–64 DOI 10.1016/j.ecoleng.2012.01.011.

Lin X, Zhu D, Lin X. 2011. Effects of water management and organic fertilization with SRI crop practices on hybrid rice performance and rhizosphere dynamics. *Paddy and Water Environment* 9(1):33–39 DOI 10.1007/s10333-010-0238-y.

Ludibeth SM, Marina IE, Vicenta EM. 2012. Vermicomposting of sewage sludge: earthworm population and agronomic advantages. *Compost Science & Utilization* 20(1):11–17 DOI 10.1080/1065657X.2012.10737016.

Mahanta K, Jha DK, Rajkhowa DJ, Manoj-Kumar P. 2012. Microbial enrichment of vermicompost prepared from different plant biomasses and their effect on rice (*Oryza sativa* L.) growth and soil fertility. *Biological Agriculture & Horticulture* 28(4):241–250 DOI 10.1080/01448765.2012.738556.

Marschner P, Kandeler E, Marschner B. 2003. Structure and function of the soil microbial community in a long-term fertilizer experiment. *Soil Biology and Biochemistry* 35(3):453–461 DOI 10.1016/S0038-0717(02)00297-3.

Martínez-Balmori D, Olivares FL, Spaccini R, Aguilar KP, Araújo MF, Aguilar NO, Guridi F, Canellas LP. 2013. Molecular characteristics of vermicompost and their relationship to preservation of inoculated nitrogen-fixing bacteria. *Journal of Analytical and Applied Pyrolysis* 104:540–550 DOI 10.1016/j.jaap.2013.05.015.

Meena HN, Shivay YS. 2010. Productivity of short-duration summer forage crops and their effect on succeeding aromatic rice in conjunction with gypsum-enriched urea. *Indian Journal of Agronomy* 55(1):11–15.

Mengi L, Sarkar NC, Verma H, Longkumer LT. 2016. Influence of different organic sources of nutrient on the productivity of upland rice (*Oryza sativa* L.). *International Journal of Bio Resource & Stress Management* 7(3):450–454 DOI 10.23910/IJBSM/2016.7.3.1483.

Murchie EH, Yang J, Hubbart S, Horton P, Peng S. 2002. Are there associations between grain-filling rate and photosynthesis in the flag leaves of field-grown rice? *Journal of Experimental Botany* 53(378):2217–2224 DOI 10.1093/jxb/erf064.

Myint AK, Yamakawa T, Kajihara Y, Zenmyo T. 2010. Application of different organic and mineral fertilizers on the growth, yield and nutrient accumulation of rice in a Japanese ordinary paddy field. *Scientific World Journal* 5(2):47–54.

Narula N, Kumar V, Behl RK, Deubel A, Gransee A, Merbach W. 2000. Effect of P-solubilizing Azotobacter chroococcum on N, P, K uptake in P-responsive wheat genotypes grown under greenhouse conditions. *Journal of Plant Nutrition and Soil Science* 163(4):393–398 DOI 10.1002/1522-2624(200008)163:4<393::AID-JPLN393>3.0.CO;2-W.
Nayak DR, Babu YJ, Adhya TK. 2007. Long-term application of compost influences microbial biomass and enzyme activities in a tropical Aeric Endoaquept planted to rice under flooded condition. Soil Biology and Biochemistry 39(8):1897–1906 DOI 10.1016/j.soilbio.2007.02.003.

Nosheen A, Bano A, Yasmin H, Keyani R, Habib R, Shah ST, Naz R. 2016. Protein quantity and quality of safflower seed improved by NP fertilizer and Rhizobacteria (Azospirillum and Azotobacter spp.). Frontiers in Plant Science 7:104 DOI 10.3389/fpls.2016.0104.

Nuemsi PPK, Tonfack LB, Taboula JM, Mir BA, Mbanga MRB, Ntsefong GN, Temegne CN, Youmbi E. 2018. Cultivation systems using vegetation cover improves sustainable production and nutritional quality of new rice for Africa in the tropics. Rice Science 25(5):286–292 DOI 10.1016/j.rsci.2018.08.003.

Pattnaik P, Mallick K, Ramakrishnan B, Adhya TK, Sethunathan N. 1999. Urease activity and urea hydrolysis in tropical flooded soil unplanted or planted to rice. Journal of the Science of Food and Agriculture 79:227–231 DOI 10.1002/(SICI)1097-0010(199902)79:2<227::AID-JSFA165>3.0.CO;2-X.

Pérez-Piqueres A, Edel-Hermann V, Alabouvette C, Steinberg C. 2006. Response of soil microbial communities to compost amendments. Soil Biology and Biochemistry 38(3):460–470 DOI 10.1016/j.soilbio.2005.05.025.

Rajeshkannan V, Sumathi CS, Manian S. 2009. Arbuscular mycorrhizal fungi colonization in upland rice as influenced by agrochemical application. Rice Science 16(4):307–313 DOI 10.1016/S1672-6308(08)60095-5.

Razavipour T, Moghaddam SS, Doaei S, Noorhosseini SA, Damalas CA. 2018. Azolla (Azolla filiculoides) compost improves grain yield of rice (Oryza sativa L.) under different irrigation regimes. Agricultural Water Management 209:1–10 DOI 10.1016/j.agwat.2018.05.020.

Rezaei H. 2013. A review of research on application of livestock manure in agricultural land of Iran. Journal of Land Management 1(1):55–68 (In Farsi).

Rodrigues MÁ, Ladeira LC, Arrobas M. 2018. Azotobacter-enriched organic manures to increase nitrogen fixation and crop productivity. European Journal of Agronomy 93:88–94 DOI 10.1016/j.eja.2018.01.002.

Sanati BE, Daneshiyan J, Amiri E, Azarpour E. 2011. Study of organic fertilizers displacement in rice sustainable agriculture. International Journal of Academic Research 3(2):134–142.

Sharma K, Garg VK. 2018. Comparative analysis of vermicompost quality produced from rice straw and paper waste employing earthworm Eisenia fetida (Sav.). Bioresource Technology 250:708–715 DOI 10.1016/j.biortech.2017.11.101.

Shirkhani A, Nasrolahzadeh S. 2016. Vermicompost and Azotobacter as an ecological pathway to decrease chemical fertilizers in the maize. Zea mays. Bioscience Biotechnology Research Communications 9(3):382–390.

Simarmata T, Turmuktiny T, Fitriatin BN, Setiawati MR. 2016. Application of bioameliortant and biofertilizers to increase the soil health and rice productivity. HAYATI Journal of Biosciences 23(4):181–184 DOI 10.1016/j.hjb.2017.01.001.
Singh YV, Singh KK, Sharma SK. 2013. Influence of crop nutrition on grain yield, seed quality and water productivity under two rice cultivation systems. *Rice Science* 20(2):129–138 DOI 10.1016/S1672-6308(13)60113-4.

Sreenivasa MN. 2012. Organic farming: for sustainable production and environmental protection. *Microorganisms in Sustainable Agriculture and Biotechnology* 23(1):55–76.

Srivastava R, Aragno M, Sharma AK. 2010. Cow dung extract: a medium for the growth of pseudomonads enhancing their efficiency as biofertilizer and biocontrol agent in rice. *Indian Journal of Microbiology* 50(3):349–354 DOI 10.1007/s12088-010-0032-y.

Taheri Rahimabadi E, Ansari MH, Razavi Nematollahi A. 2018. Influence of cow manure and its vermicomposting on the improvement of grain yield and quality of rice (*Oryza sativa* L.) in field conditions. *Applied Ecology and Environmental Research* 16(1):97–110 DOI 10.15666/aeer/1601_097110.

Tejada M, González JL. 2009. Application of two vermicomposts on a rice crop: effects on soil biological properties and rice quality and yield. *Agronomy Journal* 101(2):336–344 DOI 10.2134/agronj2008.0211.

Thakur AK, Rath S, Roychowdhury S, Uphoff N. 2010. Comparative performance of rice with system of rice intensification (SRI) and conventional management using different plant spacing. *Journal of Agronomy and Crop Science* 196:146–159 DOI 10.1111/j.1439-037X.2009.00406.x.

Tsujimoto Y, Horie T, Randriamihary H, Shiraiwa T, Homma K. 2009. Soil management: The key factors for higher productivity in the fields utilizing the system of rice intensification (SRI) in the central highland of Madagascar. *Agricultural Systems* 100:61–71 DOI 10.1016/j.agsy.2009.01.001.

Tu J, Qiao J, Zhu Z, Li P, Wu L. 2017. Soil bacterial community responses to long-term fertilizer treatments in Paulownia plantations in subtropical China. *Applied Soil Ecology* 124:317–326.

Vance ED, Brookes PC, Jenkinson DS. 1987. An extraction method for measuring soil microbial biomass-C. *Soil Biology and Biochemistry* 19:703–707 DOI 10.1016/0038-0717(87)90052-6.

Velusamy P, Immanuel JE, Gnanamanickam SS. 2013. Rhizosphere bacteria for biocontrol of bacterial blight and growth promotion of rice. *Rice Science* 20(5):356–362 DOI 10.1016/S1672-6308(13)60143-2.

Wang W, Lai DYF, Wang C, Pan T, Zeng C. 2015. Effects of rice straw incorporation on active soil organic carbon pools in a subtropical paddy field. *Soil and Tillage Research* 152:8–16 DOI 10.1016/j.still.2015.03.011.

Witt C, Gaunt JL, Galicia CC, Ottow JCG, Neue HU. 2000. A rapid chloroform fumigation–extraction method for measuring soil microbial biomass carbon and nitrogen in flooded rice soils. *Biology and Fertility of Soils* 30:510–519 DOI 10.1007/s003740050030.

Włodarczyk T, Stepniowski W, Brzezinska M. 2002. Dehydrogenase activity, redox potential, and emissions of carbon dioxide and nitrous oxide from cam-bisols under flooding conditions. *Biology and Fertility of Soil* 36:200–206 DOI 10.1007/s00374-002-0513-1.
Wu SC, Caob ZH, Lib ZG, Cheunga KC, Wong MH. 2005. Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma 125:155–166 DOI 10.1016/j.geoderma.2004.07.003.

Yadav J, Verma JP, Jaiswal DK, Kumar A. 2014. Evaluation of PGPR and different concentration of phosphorus level on plant growth, yield and nutrient content of rice (Oryza sativa). Ecological Engineering 62:123–128 DOI 10.1016/j.ecoleng.2013.10.013.

Yi-hu MA, Gu DJ, Liu LJ, Wang ZQ, Zhang H, Yang JC. 2014. Changes in grain yield of rice and emission of greenhouse gases from paddy fields after application of organic fertilizers made from maize straw. Rice Science 21(4):224–232 DOI 10.1016/S1672-6308(13)60187-0.

Zantua MT, Bremner JM. 1977. Stability of urease in soils. Soil Biology & Biochemistry 9:135–140 DOI 10.1016/0038-0717(77)90050-5.

Zhang J, Hussain S, Zhao F, Zhu L, Cao X, Yu S, Jin Q. 2018. Effects of Azospirillum brasilense and Pseudomonas fluorescens on nitrogen transformation and enzyme activity in the rice rhizosphere. Journal of Soils and Sediments 18(4):1453–1465 DOI 10.1007/s11368-017-1861-7.

Zhang X, Zhang R, Gao J, Wang X, Fan F, Ma X, Zhang CYinH, Feng K, Deng Y. 2017. Thirty-one years of rice-rice-green manure rotations shape the rhizosphere microbial community and enrich beneficial bacteria. Soil Biology and Biochemistry 104:208–217 DOI 10.1016/j.soilbio.2016.10.023.

Zhong W, Gu T, Wang W, Zhang B, Lin X, Huang Q, Shen W. 2010. The effects of mineral fertilizer and organic manure on soil microbial community and diversity. Plant Soil 326:511–522 DOI 10.1007/s11104-009-9988-y.

Zhu X, Silva CRS, Doane TA, Wu N, Horwath RH. 2013. Quantifying the effects of green waste compost application, water content and nitrogen fertilization on nitrous oxide emissions in 10 agricultural soils. Journal of Environmental Quality 42:912–918 DOI 10.2134/jeq2012.0445.