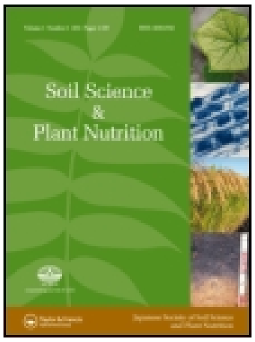

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ORIGINAL ARTICLE

Influence of Azolla (*Azolla microphylla* Kaulf.) compost on biogenic gas production, inorganic nitrogen and growth of upland kangkong (*Ipomoea aquatica* Forsk.) in a silt loam soil

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Abstract

Azolla microphylla Kaulf. (Azolla) biomass was composted to create a high nitrogen (N) organic matter amendment (Azolla compost). We examined the effect of this Azolla compost on carbon (C) and N mineralization and the production of biogenic gases, nitrous oxide (N_2O) and carbon dioxide (CO_2), in a soil incubation experiment. A pot experiment with upland kangkong (*Ipomoea aquatica* Forsk.) examined plant growth in silt loam soil treated with three levels of Azolla compost. The results showed that N_2O production from soil increased with urea amendment, but not with Azolla compost treatments. The Azolla-amended soil showed enhanced CO_2 production throughout the 4-week incubation. The Azolla-treated soils showed a 98% lower global warming potential compared to urea treatment over the 4-week incubation. However, Azolla-amended soil had higher nitrate (NO_3^-) levels compared to urea-fertilized soil at 1 week of incubation, and these were maintained until the fourth week. Soils amended with Azolla compost showed lower ammonium nitrogen (NH_4-N) levels than those in the urea-fertilized soils. The height and dry weight of upland kangkong fertilized with Azolla compost were similar to plants receiving urea fertilization. Therefore, the use of Azolla compost as a substitute for urea fertilizer would be beneficial for reducing the production of N_2O while maintaining plant growth.

Key words: Azolla, compost, greenhouse gas, kangkong, nitrous oxide.

INTRODUCTION

Applications of organic amendments to soils are useful as alternatives to chemical fertilizers for increasing crop productivity as well as improving long-term soil fertility and quality. These organic amendments may be crucial sources of fertility for small landholders who comprise the majority of farmers worldwide, and yet may lack resources such as mineral fertilizers (Herrero *et al.* 2010) or prefer organic management for environmental reasons. Incorporation of organic amendments or crop residues provides a source of readily available carbon (C) and nitrogen (N) in the soil, and subsequently influences carbon dioxide (CO_2) and nitrous oxide (N_2O) production (Padre *et al.* 2005; Lou *et al.* 2007; Toma and Hatano 2007; Kimura *et al.* 2011; Zhu *et al.* 2013). Several factors have been identified that affect the rate of N_2O emission from agricultural systems, including N amendment (Bouwman 1996; Jumadi *et al.* 2008a, 2008b; Fukumoto and Inubushi 2009), pH (Xu and Inubushi 2005), temperature (Lang *et al.* 2011), organic amendment (Lou *et al.* 2007; Vano *et al.* 2011, Thangarajan *et al.* 2013), soil moisture (Kirk *et al.* 2013; Zhu *et al.* 2013) and land use management (Jumadi *et al.* 2005; Lang *et al.* 2011; Kong *et al.* 2013). Organic amendments or fertilizers affect N_2O gas

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production in several ways: (1) the type of N [nitrate (NO_3^-), ammonium (NH_4^+) or organic N] affects N gas production during nitrification and denitrification; (2) the presence of easily decomposable organic C enhances soil respiration and biological oxygen (O_2) demand inducing low-O_2 environments and subsequent denitrification; (3) the addition of other compounds (such as salt and water) have effects on biological, chemical and physical soil processes (Velthof *et al.* 2012; Thangarajan *et al.* 2013).

Azolla (*Azolla microphylla* Kaulf.), a small floating water fern, is commonly seen in lowland rice fields and has a symbiotic association with nitrogen-fixing Cyanobacteria (i.e. *Anabaena azollae*), which inhabit the dorsal lobe of the leaves. The benefit of including Azolla in agricultural systems is primarily due to its high N fixation capacity with amounts up to 1.2 kg N ha^{-1} daily (Talley *et al.* 1977). Azolla is widely used as a biofertilizer for rice in China, Vietnam, Indonesia, Thailand and other East and South Asian countries (Cheng *et al.* 2010), and has also been used as a source of green manure and compost (Bordoloi *et al.* 2007). Although the growth and use of Azolla in South Sulawesi Province, Indonesia, has not been surveyed, based on our observations, Azolla is widespread in ponds and standing water and spreads rapidly in rice fields. Furthermore, Djojosuwito (2000) has reported that when approximately 500 kg of Azolla seed was spread in 1 ha of a rice field, the Azolla increased up to 20,000 kg ha^{-1} within 2 weeks. This suggests that Azolla has the capacity to quickly more than double, or increase up to 40-fold, the biomass of the initial seed. Composting is the controlled microbial aerobic decomposition and stabilization of organic substrates, under conditions that allow the generation of high temperatures by thermophilic microbes, to obtain an end product that is stable, free of pathogens and viable weed seeds, and suitable for crops. These biological processes also change the composition of the organic matter, particularly reducing its content of soluble organic compounds and its C to N ratio. Composting plant residues therefore affects N_2O, CO_2 and nitric oxide (NO) emissions processes compared to direct application of the same residues (Velthof *et al.* 2012; Kirk *et al.* 2013; Zhu *et al.* 2013).

Upland kangkong (*Ipomoea aquatica* Forsk.) is widely cultivated in Indonesia and has become a popular leafy vegetable compared to water spinach (*Ipomoea aquatica* Forsk.). Recently, there has been an increased demand for organic upland kangkong due to perceived health benefits. Upland kangkong often requires supplemental N for improved quality of growth. Organic amendments such as compost are important options for maintaining soil fertility and N supply.

The application of Azolla compost to soils has been proposed as a useful source of N for crops and as an amendment for increasing the organic C content of soils with low organic matter (Lumpkin and Plucknett 1980; Azmal *et al.* 1996, 1997). Some compost with high C to N ratios may promote N immobilization in soils (Norton and Schimel 2011; Zhu *et al.* 2013) and it may be appropriate to add synthetic N fertilizer to these composts in order to increase the availability of N to crops. There is, however, still little available information concerning the impact of Azolla compost applications on N availability and greenhouse gas emissions from soils. Laboratory incubation experiments were performed to understand the influence of Azolla compost on soil N levels and N_2O and CO_2 emissions. Plant growth experiments were conducted to examine the effects of the Azolla compost on the growth of upland kangkong.

MATERIALS AND METHODS

Soil analysis

The soil was sampled at a vegetable farm in Gowa district, South-Sulawesi Province, Indonesia, in 2011, and would be classified in the Entisol soil order (Soil Survey Staff 1999). Soil samples were taken in triplicate at a depth of 0–20 cm, passed through a 2-mm mesh sieve, and stored at 4°C until used. Soil and compost analyses were performed at the Soil Testing Laboratory (Department of Agriculture, South Sulawesi Province, Indonesia). Soil textures were determined by hydrometer method (Bouyoucos 1962). Soil pH was determined in a 1:5 (soil: 1M potassium chloride, KCl) using a glass electrode. Total organic N and organic C were analyzed by Kjeldahl and combustion methods, respectively (Page *et al.* 1982). Cation exchange capacity (CEC) was determined according to Burt (2004), and extractable phosphorus (phosphorus pentoxide, P_2O_5) by the Bray method (Bray and Kurtz 1945). Ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) were extracted with 2 M KCl and analyzed by colorimetric methods (Rayment and Higginson 1992). All physiochemical properties and gas production are expressed on a dry soil weight basis.

Preparation of Azolla compost

Two kilograms of Azolla (*Azolla microphyla*) plant material was collected on December 5, 2011, from a rice field in Maros district (5m above sea level, 05° 00.419' S 119° 31.219' E), South-Sulawesi Province, Indonesia. Azolla biomass was washed with distilled water three times, then dried in the oven at 55°C until the moisture content was around 50%. The dried Azolla biomass was placed in a black plastic bucket and 250 mL of molasses was added and mixed, then the bucket was covered with black plastic. The composting process continued for 1 week, and if the temperature of the material increased substantially the material was shifted into another bucket. The harvested Azolla compost was dried in the oven at 55°C, and then ground to pass a 2.00-mm sieve before analysis.

Soil incubation

A composited soil sample was preincubated at 27°C for 7 d and then kept at 55% of water holding capacity (WHC) and treated with the N source amendments. The treatments were: control (no N added), *Azolla* compost low rate (Azolla low) equivalent to 80.3 g N m^{-2} rate or about 803 kg N ha^{-1} (equivalent to urea 5 g N m^{-2} or approximately 50 kg N ha^{-1}), *Azolla* compost medium rate (Azolla med) equivalent to 160.5 g N m^{-2} rate or 1605 kg N ha^{-1} (equivalent to urea 10 g N m^{-2} or approximately 100 kg N ha^{-1}), *Azolla* compost high rate (Azolla high) equivalent to 240.8 g N m^{-2} rate or 2408 kg N ha^{-1} (equivalent to urea 20 g m^{-2} or approximately 200 kg ha^{-1}) and urea equivalent to 10 g N m^{-2} or approximately 60 µg N g^{-1} soil. Fifteen grams of soil were incubated aerobically at 27°C in sealed 120-mL bottles for 28 days in triplicate. Production of N_2O, CO_2 gases and inorganic N as ammonium and nitrate were determined for each soil sample. Every 7 d, the gases in the headspace of each bottle were taken and transferred to 22-mL vacuum vials; the incubation bottles were then aerated and resealed for later sampling. Five grams of soil was extracted for inorganic N with 1M KCl solution (1:5) and these extracts were analyzed for ammonium and nitrate colorimetrically as described above. Gas samples were sent to the Laboratory of Soil Science, Chiba University, Japan, to quantify the concentrations of N_2O and CO_2 using gas chromatographs (Shimadzu, GC 14B, Kyoto, Japan) equipped with an electron capture detector (ECD) and a thermal conductivity detector (TCD), respectively. The global warming potential (GWP) was calculated according to IPCC (2007) which was done as sum of N_2O production during incubation time and then converted to factor as 1 kg N_2O is equivalent to 298 kg CO_2 at 100 years' time horizon. The mitigation potential was assessed as the percentage of reduction in N_2O production from soils treated with urea compared to those treated with Azolla compost.

Upland Kangkong growth

Pot experiments were conducted in the field at the Biology Department Farm, Makassar State University, during January to March 2012. The randomized block experimental design consisted of four treatments plus one control (check), each with three replicates; the experiment was repeated twice (30 pots total). The polyvinyl chloride (PVC) pots had an inner diameter of 22.5 cm and a depth of 20.5 cm. The treatments and soil characteristics were the same as those used in the incubation experiment. Azolla compost and urea were applied at one time by spreading and incorporation to the surface soil 5 d after sowing seeds of upland kangkong. The growth of upland kangkong was determined after 35 d. Plant height (cm) was measured weekly and, at the end of the growth period, the aboveground plant material was harvested and the dry weight was determined (Singla *et al*. 2013). Statistical analysis was performed using SPSS version 16.0 (IBM Corp 2008). Post-hoc comparisons were examined by least significant difference (LSD = 0.05).

RESULTS

Soil and Azolla compost characteristics

The soil was a silt loam with initial physicochemical properties as follows: texture; 26% sand, 47% silt, and 27% clay; pH (KCl 1:5) 4.1; total organic C 1.47%; total organic N 0.18% and C/N ratio 8.2; CEC 28.13 ($cmol_c$ kg^{-1}); extractable phosphorus 50 mg P_2O_5 g^{-1} soil; NH_4^+-N 4.08 µg g^{-1} dry soil; and NO_3^-N 7.51 µg g^{-1} dry soil. These characteristics are typical for upland soils under agriculture with the use of chemical fertilizers. The total organic C and N of Azolla compost were 27.7 and 2.7%, respectively, resulting in a C/N ratio of 10.

Production of N_2O and CO_2, global warming potential and inorganic N in a silt loam soil

The production of nitrous oxide (N_2O) gas was significantly increased by the addition of urea compared to the Azolla compost-amended or control soils. The Azolla amendment did not significantly affect the N_2O production during the 4-week incubation period (Fig. 1). N_2O production in all treatments peaked at the 3-week sampling, except for the high-level Azolla compost which was highest at the 1-week sampling and then decreased. The urea addition exhibited the highest production of N_2O for the third week of the incubation (890 ng g^{-1}), then decreased to 234 ng g^{-1} in the fourth week.

The production of CO_2 from the treated soils is given in Fig. 2. Amendments of Azolla compost and urea remarkably enhanced CO_2 production. In the first week of incubation, the soil respiration was significant higher for the medium- and high-level Azolla compost treatments. During the third and fourth weeks, the Azolla high and urea-treated soils both showed elevated CO_2

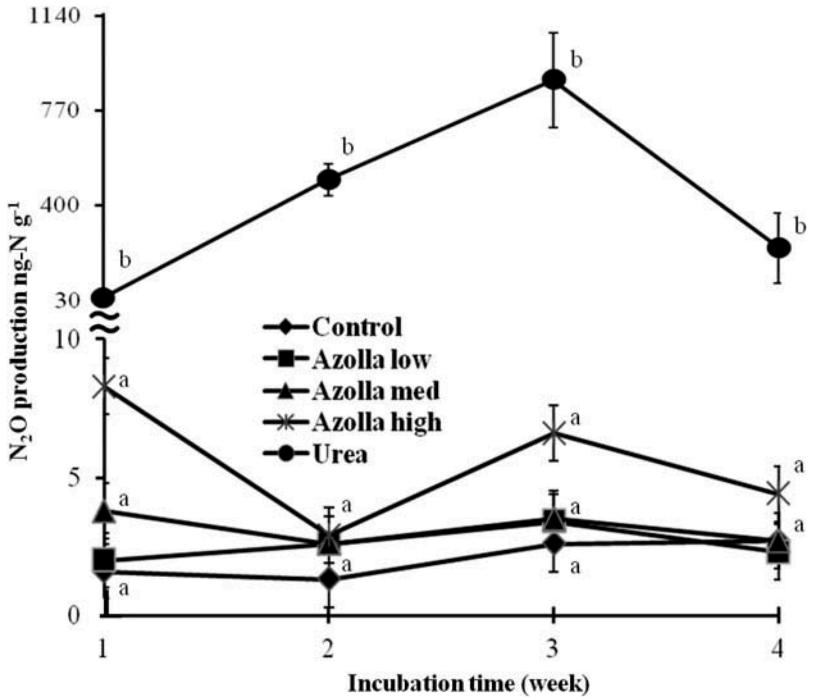

Figure 1 Production of nitrous oxide (N_2O) during soil incubations sampled weekly. Value followed by different letters indicate significant different (least significant difference, LSD; $P < 0.05$). The error bars indicate the standard deviations. N, nitrogen.

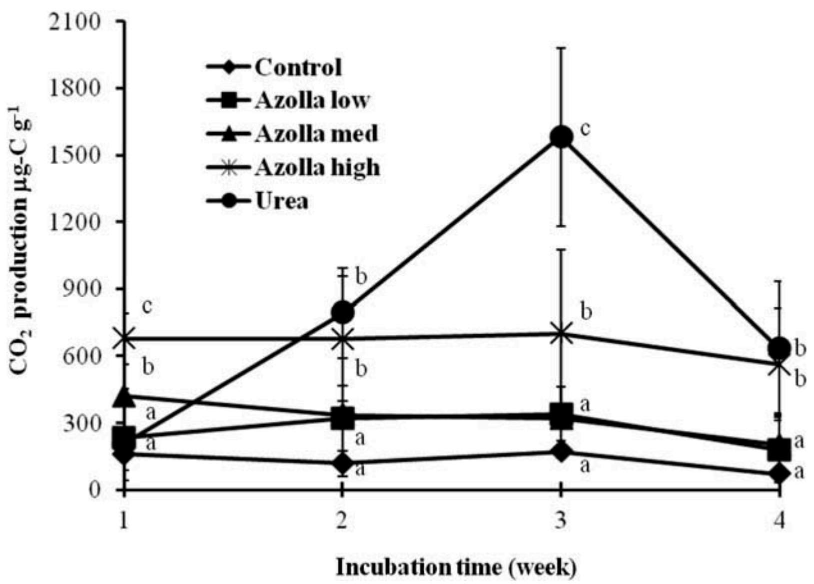

Figure 2 Production of carbon dioxide (CO_2) during soil incubations sampled weekly. Value followed by different letters indicate significant different (least significant difference, LSD; $P < 0.05$). The error bars indicate the standard deviations.

production. Soil treated with urea significantly contributed to GWP (966 kg CO_2 ha^{-1}) compared to other treatments: Azolla low with 6 kg CO_2 ha^{-1}, Azolla medium with 7 kg CO_2 ha^{-1} and Azolla high with 13 kg CO_2 ha^{-1}. The results also showed that the reduction of sources to atmospheric radiative forcing of CO_2 gas was approximately 98–99%. Cumulative levels of inorganic N are shown in Tables 1 and 2. During the first week, the Azolla medium- and high-level amendments showed elevated nitrate levels. During the second and third

Table 1 Levels of ammonium (NH_4^+) (µg N g^{-1}) accumulated during incubation of the silt loam soil

Treatments	Concentration of NH_4^+ (µg N g^{-1}) incubation time (week) 1	2	3	4
Control	4.1^{a}	1.8^{a}	1.9^{a}	1.5^{a}
Azolla low	4.5^{ab}	2.6^{a}	2.5^{ab}	2.5^{a}
Azolla med	5.1^{ab}	2.7^{a}	3.5^{ab}	2.6^{a}
Azolla high	6.4^{b}	6.3^{b}	4.1^{b}	3.5^{a}
Urea	19.8^{c}	15.2^{c}	10.2^{c}	6.1^{b}

N, nitrogen. Values followed by different letters in each column indicate significant differences (least significant difference, LSD; $P < 0.05$).

Table 2 Levels of nitrate (NO_3^-) (µg N g^{-1}) accumulated during incubation of the silt loam soil

Treatments	Concentration of NO_3^- (µg N g^{-1}) incubation time (week) 1	2	3	4
Control	46.9^{a}	41.6^{a}	57.1^{a}	68.9^{a}
Azolla low	72.8^{ab}	89.4^{b}	84.3^{b}	126^{b}
Azolla med	74.3^{b}	90.2^{b}	88.5^{b}	146^{b}
Azolla high	82.8^{b}	98.6^{b}	116^{c}	158^{b}
Urea	63.0^{ab}	79.4^{b}	166^{d}	33.5^{a}

N, nitrogen. Values followed by different letters in each column are significantly different (least significant difference, LSD; $P < 0.05$).

weeks, all Azolla compost treatments and the urea treatment had elevated nitrate, which persisted for the Azolla treatments through the incubation period. By the fourth week, the urea treatment nitrate levels were not significantly different from those of the control soil. Soils amended with Azolla compost showed lower NH_4-N levels than those in the urea-fertilized soils.

Growth of upland kangkong

The height and dry weight of kakong plants revealed that both Azolla compost and urea can be used to increase the plant growth of upland Kakong as compared to growth in non-amended soils (Fig. 3 and Table 3). Azolla compost treatments enhanced the height of plants starting in the third week after sowing and it remained significantly higher through harvest. At the end of the growth period, the dry weight of the plants receiving Azolla compost was not significantly different than the control or the urea treatment, while the urea-fertilized plants were significantly higher than the control.

DISCUSSION

The Azolla material rapidly composted due to thin leaves and low recalcitrance. Several studies reported that the C/N values of mature composts are between 10 and 15 (Rynk 1992). Zbytniewski and Buszewski (2005)

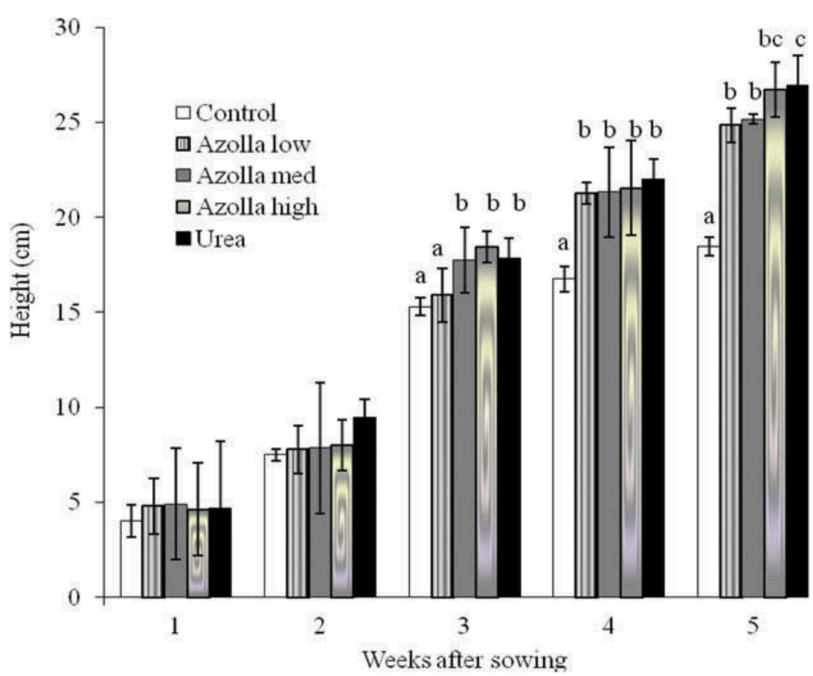

Figure 3 Height (cm) of upland kangkong (*Ipomoea aquatica* Forsk.) grown in pots. Treatments are: control (no amendment), Azolla Compost low (80.3 g m^{-2}), Azolla Compost medium (160.5 g m^{-2}), Azolla Compost high (240.8 g m^{-2}), Urea (10 g N m^{-2}). Different letters refer to significant differences (least significant difference, LSD; $P < 0.05$).

Table 3 Plant biomass (dry weight, grams per pot) of aboveground upland kangkong (*Ipomoea aquatica* Forsk.) studied in a pot experiment. Soil treatments are the same as for Fig. 1. Different letters refer to significant differences (least significant difference, LSD; $P < 0.05$)

Treatments	Plant biomass (dry weight) (gram pot^{-1})
Control	4.77^{a}
Azolla low	5.13^{ab}
Azolla med	5.30^{ab}
Azolla high	5.38^{ab}
Urea	5.42^{b}

Soil treatments are the same as for Fig. 3. Values followed by different letters in each column are significantly different (LSD, $P < 0.05$).

reported that a C/N ratio of about 15 expresses stabilization of composting mass, and one below 12 indicates a high degree of compost maturity. In addition, Komilis and Tziouvaras (2009) and Komilis *et al.* (2011) also suggested that a C/N ratio of 10 indicates mature composts. Hence with a C/N ratio of 10, we judged the maturity and stability of the *Azolla* compost as suitable for our research purposes.

The high CO_2 and N_2O release from the urea treatment may indicate the loss of N through nitrification or denitrification processes in the urea treated soils. The amendment of organic matter can substitute for or supplement mineral N fertilizer in agricultural systems; however, the management of the timing of nutrient release remains a challenge. Soil organic matter (SOM) content and texture are important factors affecting C and N mineralization under constant soil moisture (Zhu *et al.* 2013). The N and C available to soil from an amendment are influenced by a complex interaction of amendment characteristics, soil characteristics and the rate of application (Zhu *et al.* 2013).

The Azolla compost used was produced using the addition of a readily decomposable soluble organic C (molasses) which promoted the immobilization of the available N into microbial biomass and products. Therefore, the Azolla compost used with a relatively low C/N of 10 should release N relatively rapidly into the soil. This addition of readily decomposable organic matter increased microbial activity as indicated by CO_2 production. Compost addition typically leads to the buildup of soil organic matter and the formation of macro and micro aggregates (Shibahara and Inubushi 1997; Zaman *et al.* 2002).

The C/N ratio of amendments may also be useful in predicting N mineralization (Norton and Schimel 2011) and subsequent production of N_2O through nitrification and denitrification processes (Toma and Hatano 2007). In all treatments, the cumulative N_2O and CO_2 productions during 4 weeks of incubation were linearly correlated with total mineral N concentration (NH_4^+ and NO_3^-) with $R^2 = 0.99$ and $R^2 = 0.98$, respectively. This implies that N_2O and CO_2 gas production was mainly related to N mineralization. Our observations agree with a previous report (Sánchez-Martín *et al.* 2008) where increasing N_2O production under aerobic condition was not correlated with the amount of dissolved organic matter but was related to the NH_4^+-N levels in the soil. Soils with different treatments showed different contributions to GWP. And seems that mostly GWP from the soils during the 4 weeks' incubation were produced when NH_4^+ content in soil was higher particularly in soil amended with urea. The total GWP from soils was within the range reported by Yu and Patrick (2004) at 8.6–250.6 kg CO_2 ha^{-1} and Mu *et al.* (2013)'s range of 1.401–3.596 g CO_2 m^{-2}, while total GWP at urea amendment was highest. Therefore, there is much mitigation potential for N_2O production by using Azolla as a replacement for chemical fertilizer. During this experiment, the soil was at 55% of WHC, favoring nitrification over denitrification processes, and it is possible that the majority of N_2O was produced by nitrifiers. Nitrification is stimulated by high concentrations of NH_4^+ and does not require an organic C source (Norton and Stark 2011). The soil in this experiment had received long-term application of N fertilizers that may have resulted in increased potential for rapid nitrification. The significantly higher N_2O production observed in soil treated with urea may also be explained by higher NH_4^+ leading to increased nitrification in urea-treated soil compared to those treated with Azolla compost, even with equivalent amounts of N applied (Tables 1 and 2). Therefore, a significant increase in cumulative N_2O production in urea versus Azolla treatments was observed even though both treatments had similar NO_3^- contents. The higher NH_4^+ content in urea-treated soils throughout the incubation suggests these soils had favorable conditions for nitrification.

Nitrate N is the predominant form of N that is available in well-aerated soils, except for a short period after the addition of fertilizers containing NH_4^+ or urea (Stevenson and Cole 1999), and this experiment followed this generalization. Inorganic N pools were dominated by nitrate overall, with transient increases in the ammonium pool due to urea and its subsequent hydrolysis. Increases in the ammonium pool due to the Azolla high-level compost occurred during the first 3 weeks but were not evident by the fourth week. In contrast, the released ammonium from both compost and urea was nitrified and increased the nitrate pool during the incubation, although the timing of release was distinct for compost versus urea.

The mineralization processes of organic N and C are related to one another (Stevenson and Cole 1999). The mineralization of soil organic matter in Azolla compost treatments provides a continuous release of ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) (Tables 1 and 2). As expected, Azolla compost-amended soil released NH_4^+ more slowly compared to the release of urea N to soils. Previous studies have also suggested that processes related to nitrification, including nitrifier denitrification, are a major source of N_2O emissions in fertilized and compost-amended soils (Zhu *et al.* 2013). Alternatively, increased ammonium also increases the availability of the nitrification product, nitrite or nitrate, that may be denitrified to N_2O in low-O_2 microsites in the soil.

Decomposition of the Azolla composts in the soil, as indicated by CO_2 production, was directly related to the amount of Azolla compost applied (Fig. 2). Azolla compost applications at the medium and high rates resulted in increased CO_2 production, reflecting readily available organic matter (Miyittah and Inubushi 2003). Increasing soil respiration rates would increase consumption of O_2 by microbes and may create microsites with the transiently anaerobic conditions required for denitrification. This is a probable explanation for our observation that N_2O production was significantly higher during the third week. This time period coincided with higher respiration combined with higher NO_3^- availability (Table 2), likely enhancing denitrification as well as nitrification sources of N_2O.

Sánchez-Martín *et al.* (2008) suggested that organic amendments instead of inorganic fertilizer may reduce the emission of N_2O and NO from agricultural fields. Rao *et al.* (1995) reported lower gaseous N losses from incorporated green manures than from urea applied in three splits in their field experiments, probably because mineralization of green manure N provided for more continuous N availability than urea application. Ding *et al.* (2013) reported that there was a significant difference between the nitrogen (N), phosphorus (P), and potassium (K) (NPK) fertilizers and organic matter and suggested that increasing Soil Organic Carbon (SOC) by compost application, then partially increasing N supply to crops versus adding inorganic N fertilizer alone, may be an effective measure to mitigate N_2O emissions from arable soils in the North China plain region.

Azolla compost treatments enhanced the height and dry weight of plants. Compost contains a proportion of available organic C and N that can increase soil fertility (Rao *et al.* 1995; Warman 2005; Diacono and Montemurro 2010; Vano *et al.* 2011) as well as inducing the activity of enzymes and microbial biomass soil (Zaman *et al.* 2002; Ros *et al.* 2006). Therefore, Azolla compost would stimulate soil microbes and increase nutrient recycling and decomposition in soil. Amir *et al.* (2012) also observed similar results in a similar pot experiment examining the growth of spinach (*Amaranthus tricolor* L.). They observed that Azolla compost treatments and urea fertilizer both increased the height and dry weight of spinach plants.

Our observations suggest that using Azolla compost as an amendment has the potential to reduce N_2O emissions when compared to urea fertilization. Azolla compost application may be particularly useful for vegetable crops in organic farming systems. In addition, Azolla treatments showed a reduction of atmospheric radiative forcing compared to urea-amended soils. Azolla compost is a viable amendment considering the agronomic benefits provided, while decreasing the likelihood of N_2O production under moderate moisture conditions. Further study is needed for long-term evaluation in organic farming systems as examined by Nagano *et al.* (2012).

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