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Research article

EARTHWORM *PONTOSCOLEXCORETHRURUS* AND NITROGEN MINERALIZATION RATE IN INCUBATION EXPERIMENT WITH DIFFERENT QUALITY ORGANIC MATTERS FROM SUGARAGRO-INDUSTRY WASTE

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ABSTRACT: To investigate the effect of earthworm inoculation on N mineralization rates of different quality organic matters from sugar agro-industry waste, we conducted a green house incubation experiment for 14 weeks in pots containing 7 kg dry soil. There are 12 treatment combinations.Factor I waswith (P) and without inoculation of *P.corethrurus* (N). Factor II was the type of OM which consisted of six levels: (1) without application of OM (control), (2) cow manure (CM), (3) filter cake (FC), (4) sugarcane trash (ST), (5) a mixture of CM + FC and (6) a mixture of CM + ST. The treatments were arranged in a factorial randomized block design with three replications. The amount of released N-$NH_4^+$and N-NO_3^- or mineral N was measured during incubation time from 1, 2, 4, 8, and 14 weeks after inoculation of earthworms. There was a significant effect of earthworm inoculation on N mineralization rate of different quality organic matters. Overall, rate of net N mineralization were higher in treatments with earthworm inoculation than without earthworm inoculation and the magnitude of the increase appears to dependent on the quality of organic matters. The largest difference were seen on ST and CM+ST treatments with the increase by 90 % and 157 % and the constant of N mineralization rate by 0.0147 and 0.0180 week^{-1} for the treatment with earthwormsinoculation.These results suggested that application of sugar agro-industry waste although having low quality can improve soil N availability in sugarcane land when aided by *P.corethrurus* activity.

Key words: Earthwoms *Pontoscolexcorethrurus*, Nitrogen, Mineralization, incubation, sugar agro-industry waste

INTRODUCTION

Conventional management in sugarcane cultivation in Indonesia relies heavily on high nitrogen inputs. To ensure that the nitrogen requirements of high-yielding sugarcane plant, sugarcane farmer usually use ammonium sulfate as inorganic fertilizer in a high dose by 1000-2000 kg ha^{-1}. The exceed application is economically wasteful and potentially damaging to the environment. To reduce the use of inorganic fertilizer and conserve the resources and the environment, it is important to know the soil fauna contribution to N supply, especially in sugarcane plantation land which Indonesia as a sugar producing country.

Many studies on earthworms carried out in the humid tropics have shown that earthworm improve structure (measured by increased infiltration and resistance to erosion through the formation of aggregates), activate nutrient release from soil organic matter, and physically protect such organic matter in the structure of their casts (Pashanasi *et al*., 1992; Lamande *et al*., 2003). Studies on earthworms in tropical soils were done by Fragoso *et al.,* (1997), Kale, (1998), Singh, (1997), Kaushal *et al*., (1999), Bisht *et al*., (2003), and Dewi *et al.,* (2006), however there were not as many as in temperate climate areas. Positive effects of earthworms on soil fertility have been documented by several authors (Lee, 1985; Helling and Larink, 1998; Peres *et al.,* 1998). There is considerable evidence that earthworms can accelerate the disappearance of organic litter from soil surface (Bohlen *et al.,* 1997) and stimulate microbial activity on the mineralization of nitrogen compounds in soil (Bityutskii *et al.,* 2007). The effect of earthworms on the nitrogen mineralization rate in the soil is due to various organic matter from sugar agro-industrywaste induced by the earthworm *P.corethrurus* poorly studied. In Indonesia, very few studies were conducted on the role of earthworm communities in sugarcane land, because the researchers assumed that sugarcane grown in dry-land was unsuitable habitat for earthworms.

Nurhidayati *et al*. (2011) reported that there were two earthworm species found in sugarcane land namely *Pontoscol excorethrurus* (Glossoscolecidae) and *Pheretima minima* (Megascolecidae). The former was more dominant.However, agricultural activities such as plowing, soil treatment, fertilization and application of chemical pesticides in sugarcane cultivation have a negative impact on the earthworms.

The objectives of this studyis to investigate effect ofearthworm *P.corethrurus*inoculation onsoil concentrations of mineral nitrogen and N mineralization rates of various organic matters with different quality from sugar agro-industry waste.This study is an incubation experiment for 14 weeks used soil samples from sugarcane land and applied with different quality organic matters from sugar agro-industry waste.

MATERIALS AND METHODS

Soil characterization

Soils were sampled from the surface 20 cm layer of sugarcane land at Sempol village, Pagak District, Malang Regency, East Java, Indonesia. The soil was low in organic carbon (1.16 %), with pH 5.1 (Acid), low in total N (0.16%), low in phosphorus (9.17 $mgkg^{-1}$), medium in available K (0.534me/100 g soil), medium in CEC (23.23 me/100 g soil). The soil had 26 % sand, 48 % silt, and 26 % clay.

Organic materials characterization

Organic materials which used in this research are from sugar agro-industry waste that are filter cake (FC) and sugarcane trash (ST). This study also used cattle manure (CM) as organic matter with high quality and the mixture of CM+FC and CM+ST. The dry sugarcane trash was ground and decomposed for two weeks. The filter cake and cattle manure were dried and ground. The materials were analyzed for total C (Walkley Black method), total N (Kjeldahl method), cellulose, lignin and ash (Goering and Van Soest, 1970), polyphenols(Folin-Denis method), and gross energy (Bomb Calorimeter method). The results of analysis were presented in Table 1.

Table 1. The chemical composition of organic matter on dry weight basis

Organic matter	C-org (%)	Total N (%)	C/N	Lignin (%)	Ash (%)	Cellulose (%)	Polyphenol (%)	Gross Energy (Kcal/kg)
CM	16.17	1.94	8.3	12.32	13.26	30.34	0.26	1010.63
FC	20.15	1.98	10.2	19.88	20.46	40.22	1.14	1089.54
ST	28.14	0.81	34.7	13.30	10.22	40.09	2.01	3027.78
CM+FC	19.15	1.68	11.4	16.46	11.46	37.45	1.42	1120.14
CM+ST	20.43	1.32	15.5	12.03	8.22	33.25	1.12	1353.54

Treatments

The treatments were arranged in factorial block randomized design. The first factor consisted of two treatments, that is with inoculation of earthworm *P.corethrurus* (P) and without *P.corethrurus* inoculation (N), The second factor is organic matter source that consist of five kinds of organic matter with different quality, that is, cattle manure, CM (O_1), filter cake of sugar mill, FC (O_2), trash of sugarcane, ST (O_3), mixture of CM+FC (O_4), and mixture of CM+ST (O_5). The two factors were obtained from 10 treatment plus two control, that is, without inoculation and no organic input (NO_0) and with inoculation and no organic input (PO_0). Each treatment was replicated three times.

Application of organic matters

The soil samples were air-dried, ground and passed through a 2-mm sieve. A 7 kg soil was placed in a plastic pot (35cm x 27cm x15 cm). Each plastic pot was layered by sponge (2 cm thickness) and a set of polyvinyl chloride tube (3 cm in diameter and 5 cm in high). Above of the tube was layered by plywood (0.5 cm thickness). The organic matters were added to the soil at rate of 5 g kg^{-1} on dry weight basis. They were mixed thoroughly and the moisture content brought to about 50% before inoculation and incubation.

Earthworm Inoculation

Earthworm *P.corethrurus* which was obtained from coffee plantation was inoculated into each plastic pot after acclimatization as 20 individuals per plastic pot. After inoculation, the top of the plastic pot was wrapped around by a black cloth to allow a dark condition. Each plastic pot was placed at green house accordance with treatments sketch in experimental design. Two control treatments was included. During measurement of N-mineral release, soil water content was maintained as 50%.

Measurement of N-mineral release

After 1, 2, 4, 8, and 14 weeks of incubation, the soil of each treatments was sampled and measured water content. Two grams of soil from each plastic pot was removed and extracted with 20 ml of 1 N KCl. The NH_4^+-N and NO_3^--N was then analyzed by Kjeldahl distillation method used reagent of MgO and Devarda's alloy.

Distillate was collected in a conical bottle containing 5 ml of boric acid until 30 ml volume and titrated with 0.002 N H_2SO_4 until the color changes from green to pink. The amount of NH_4^- and NO_3^- expressed in mg kg^{-1} was calculated with the formula: (ml sample – ml blank) x N H_2SO_4 x 14 x 10^3 x (% water content+100)/100. Net rate of ammonification, nitrification, and N mineralization in the soil were calculated as difference between the accumulation of NH_4^+-N, NO_3^--N or total N-mineral (NH_4^+ + NO_3^-) of each treatment with control (no organic matter input), during incubation time. Total N-mineral released was calculated by summing the amount of nitrogen mineralized (or immobilized) for all of the incubation periods.

Statistical analysis

Data at each sampling time was statistically analyzed by using analysis of variance (ANOVA) a factorial block randomized design at level $P \leq 0.05$ and LSD testat level $P \leq 0.05$ to compare among the treatments using Minitab Vers.14 software.For statistical analysis of data (correlation and charts) Microsoft Excel was used.

RESULTS

Soil concentrations of mineral nitrogen

Overall, the treatments inoculated *P.corethrurus* had significantly higher concentrations of NH_4^+-N and NO_3^--N than the treatments without *P.corethrurus* inoculation. In the treatments inoculated *P.corethrurus*, peak concentration of NH_4^+-N occurred just on 14 week of incubation time, exception for the mixture of CM+ST occurred on 2 week of incubation time (Fig. 1).

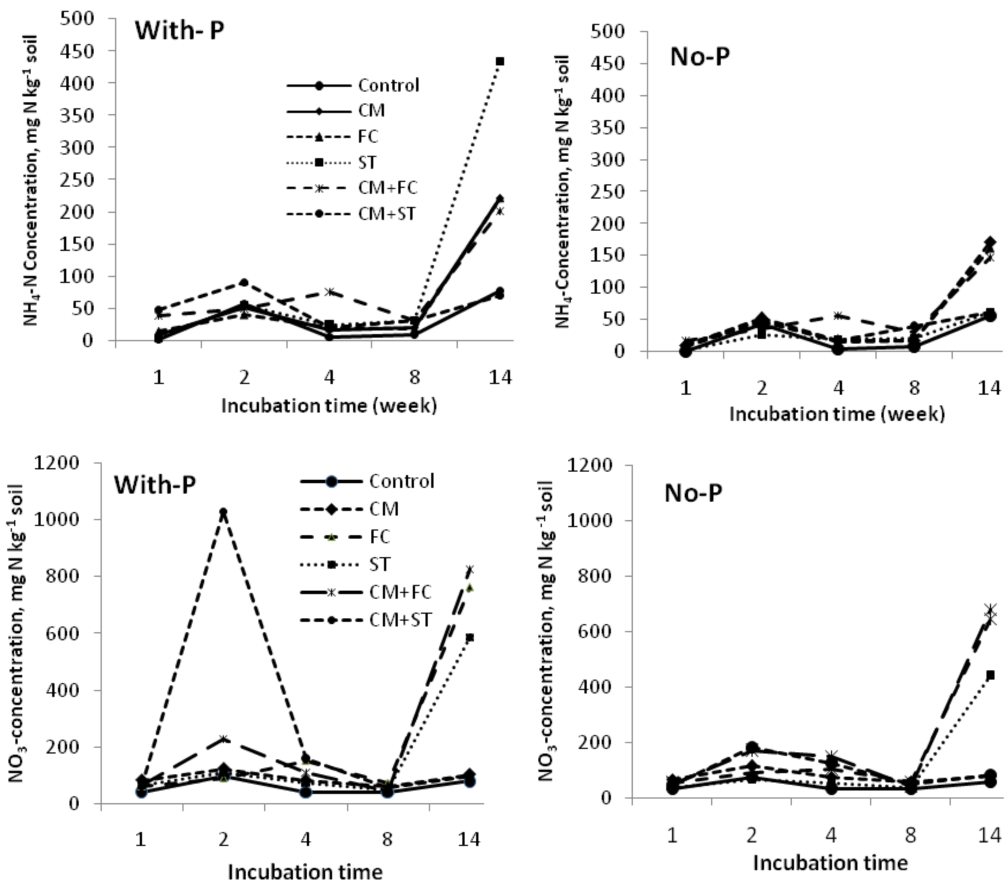

Figure 1. Soil concentration of NH_4^+-N and NO_3^--N on the various organic matter application and earthworm inoculation (Note: With-P= with inoculation *P.corethrurus*; No-P= without inoculation *P.corethrurus*)

The average increase of NH_4^+-N concentration for each incubation time (1, 2, 4, 8, 14 weeks) were 83, 33, 27, 26 and 149%, respectively, while for NO_3^--N concentration were 43, 30, 22, 25 and 29 %, respectively when compared with the no-*P.corethrurus* inoculation treatments. Differences in the quality of organic matter and earthworm inoculation significantly affected on the amount of cumulative NH_4^+, NO_3^- and N-mineral that is released during the incubation period of 14 weeks.

The highest amount of NH_4^+ release was found on the application of sugarcane trash, whereas the highest amount of NO_3^- and N-mineral release was found on the applications of mixture sugarcane trash+cattle manure with earthworm inoculation. The highest NH_4^+, NO_3^-, N-mineral was found on the application of mixture CM+FC without earthworm inoculation (Fig.1). The highest increase average of NH_4^+-N concentration for each incubation time was found on the sugarcane trash application by 192%, while for NO_3^--N concentration was found on mixture CM+ST application by 115 %. These results indicate that the presence of earthworm inoculation *P. corethrurus* can accelerate the N mineralization of low quality organic matter.

Net rates of ammonification, nitrification, and N mineralization

Overall, net rates of ammonification, nitrification and N mineralization were higher in the earthworm inoculation treatment than no inoculation (Fig.2& 3). Net rates of ammonification and nitrification were significantly different among various organic matter applied. During incubation time, net NH_4-N and net NO_3-N increase until 14 weeks incubation time, exception for the CM+ST application. In the CM+ST application, peak of net NO_3-N occurred in two weeks of incubation time (Fig.2).

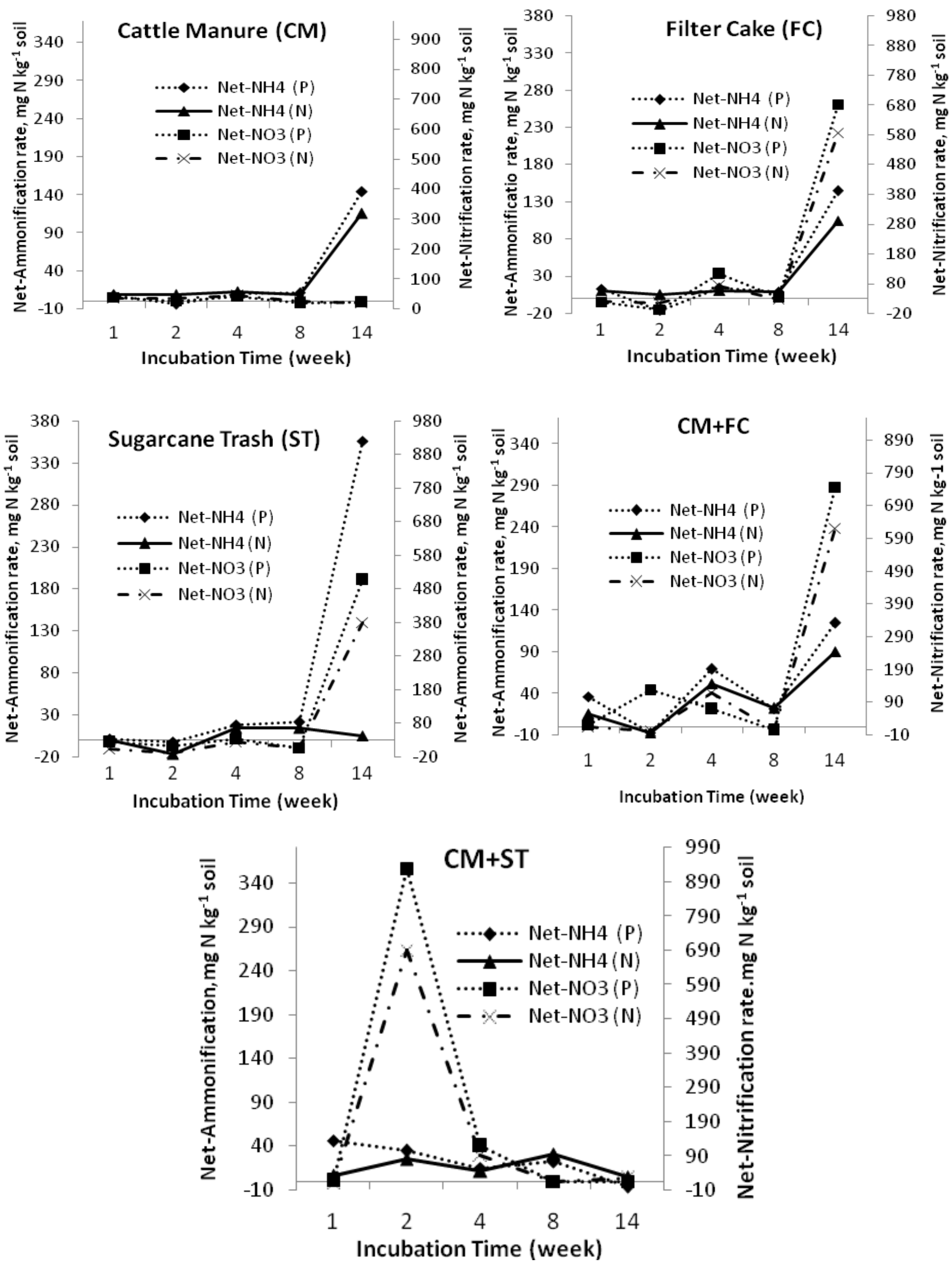

Fig.2. Net rates of ammonification and nitrification of various organic matters in the treatment with earthworms inoculation (P) and without inoculation (N).

A significant difference in the pattern of N-mineralization between without and with earthworms inoculation was showed in the treatments with earthworm inoculation had higher net mineralization than without inoculation. The peak of net N mineralization occurred in 14 weeks of incubation time, exception application of a mixture of CM+ST occurred at two weeks of incubation time with adramatic increase in the release of N-mineral (Fig. 3). A high quality organic matter (CM) witha high N content and low in C/N ratio showeda constant net mineralization rate, while other organic matters showed the N-mineralization rates increased with increasing incubation time. A significant difference also occurred in the application of sugarcane trash, which in the treatment with inoculation had ahigher amount of N-mineral at late incubation period (14 weeks) than without inoculation *P.corethrurus* (Fig. 3).

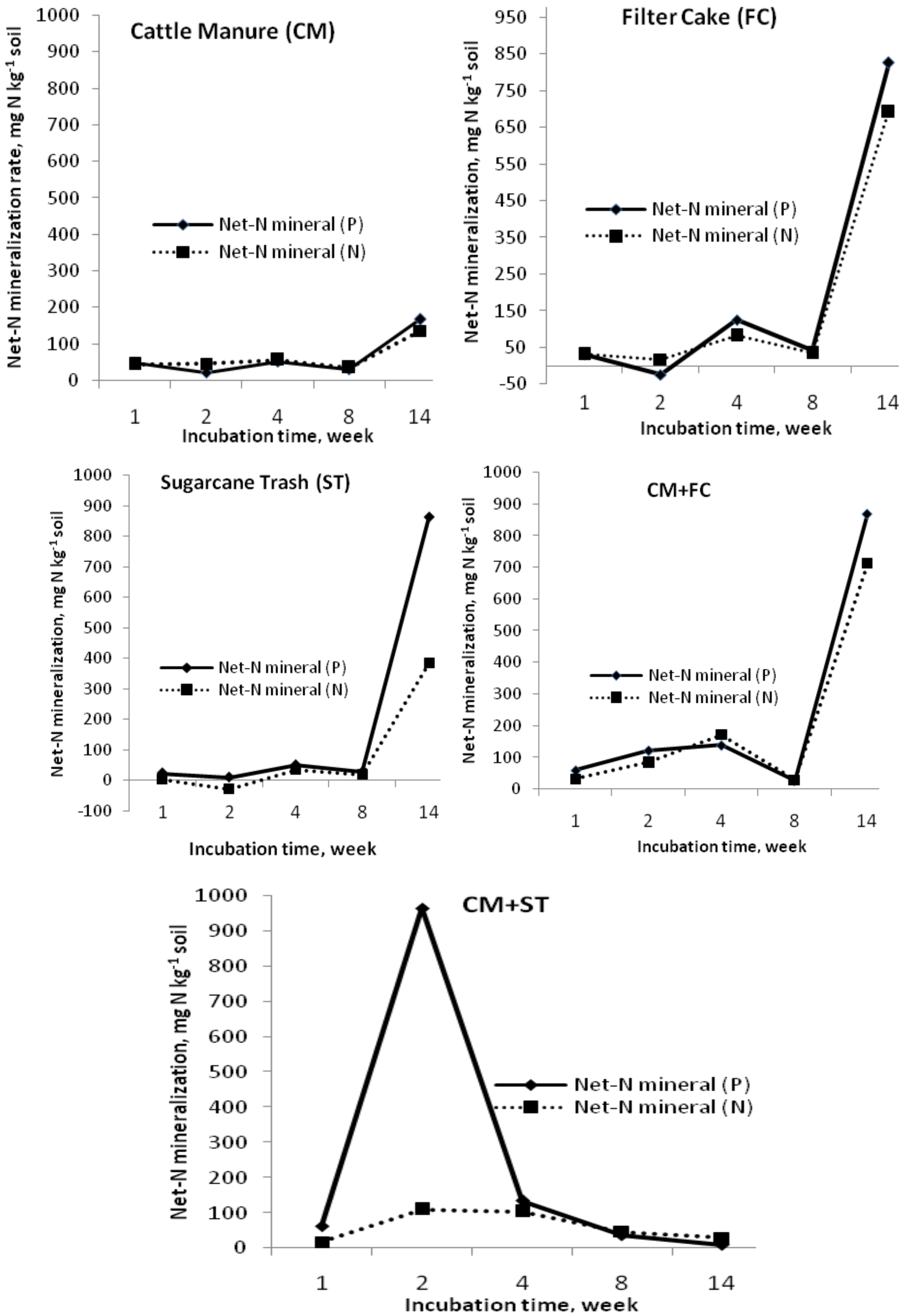

Fig.3.Net rate of N mineralization of various organic matters in the treatment with earthworms inoculation and without inoculation.

The results of the calculation of the mineralization rate constant (k) during the incubation period of14weeksusing the equation Wieder and Lang (1982) showed that the application of low quality organic matter such as sugarcane trash (ST) alone or in a mixturehadtheslowN mineralizationrateonthe treatmentwithoutearthworm inoculation. However, with earthworm inoculation increased N mineralization rate of low quality organic matterindicatedby thehighermineralizationrateconstant than without inoculation. Thisresult showedthe role ofearthwormsin acceleration ofthe Nrelease oflow qualityorganic matters. The highestrate of Nmineralizationwas foundin thetreatment ofmixture CM+FCon the treatmentwithoutearthworm inoculation, whileonthe treatmentwithearthworminoculation, the highestN mineralizationratewas found onthe treatment of mixtures CM+FC and CM+ST (Table 2).

Table2. N Mineralization rates constant of various organic matters with and without earthworm inoculation treatments.

Types of organic matters	N Mineralization rates constant (k), (minggu^{-1})	
	Without earthworms	With earthworms
Cattle Manure (CM)	0.0046 a	0.0047 a
Filter Cake (FC)	0.0127 c	0.0154 b
Sugarcane Trash (ST)	0.0061 b	0.0147 b
CM+FC	0.0157 d	0.0187 c
CM+ST	0.0046 a	0.0180 c
LSD 5 %	0.0012	0.0012

Interaction between earthworm inoculation and type of organic matter significantly affected the total N-mineral released. A high quality organic matter (C/N = 8.3) had the lowest amount of N- mineral release, while moderate quality of the organic matters (a mixture of CM+FC and CM+ST) with a C/N ratio of 11.4-15.5 released the highest N-mineral for 14 weeks of incubation time on the treatment with earthworm inoculation (Fig.4).

The percentage of increase of the N-mineral released on the treatment with earthworm inoculation compared with no earthworm inoculation for each organic matters by 37% (control), 18% (CM), 22% (FC), 90% (ST), 22% (CM + FC), and 157% (CM+ST) with an average magnitude of the increase as 112.35, 119.98, 265.22, 680.22, 306.91 and 1020.31 mg N kg^{-1}soil. This suggests that *P.corethrurus* earthworm can accelerate N mineralization of low (ST) and medium (CM+ST) quality organic matters (Fig. 4).

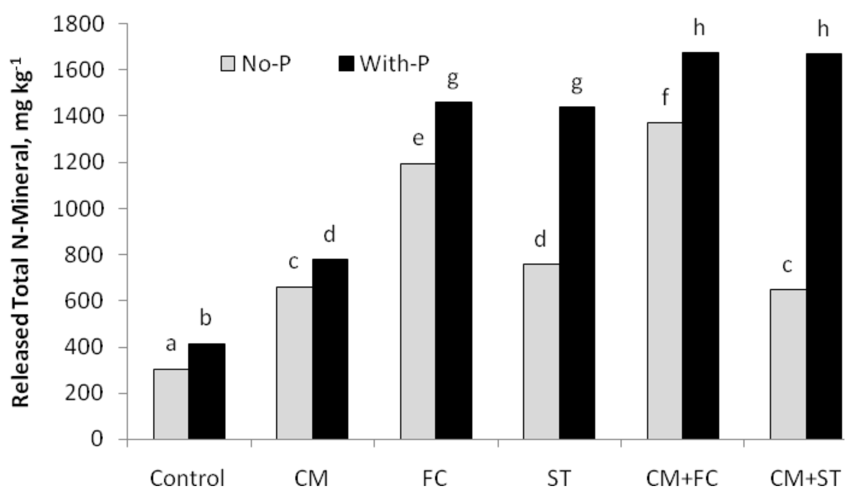

Figure 4. The total N-mineral of each organic matter released on the treatment with (With-P) and without inoculation *P.corethrurus* (No-P).

DISCUSSIONS

Our results suggest that *P.corethrurus* which was found in sugarcane land can increase the short-term availability of N-mineral by reducing the net immobilization into microbial biomass. There is no indication of immobilization of nitrogen in microbial biomass in the incubation initial period after organic matter addition. The net immobilization just occurred on the filter cake addition with earthworms inoculation and on the sugar cane trash addition without earthworms inoculation at two weeks of incubation time.

There was significantly an increase in N-mineral concentration in the treatments with earthworms inoculation, and the N-mineral concentration in the treatment with earthworms inoculation was higher over the incubation period than in the treatments without earthworm inoculation. Sublér*et al.* (1998) reported greater net cumulative net Nitrogen mineralization in the agroecosystem with the earthworm addition than the earthworm reduction enclosures. There is the other good evidence for increased microbial immobilization of N in the earthworm reduction enclosures. Blair *et al.* (1997) reported greater microbial biomass N in the same earthworm reduction enclosures than in the earthworm addition and unmodified enclosures. Bityutskii*et al.* (2007) reported that increase in net ammonification and nitrification occurred because of the positive indirect impact of the earthworm excreta on nitrification process is ensured by the presence of ammonium ions in the excreta.

Our results are contradictory with the common N mineralization which the higher N content of organic matter, the higher mineralization rate. In contrast, the higher lignin, cellulose, and polyphenol, the slower N mineralization rate.Cattle manure are a high quality organic matter (C/N ratio = 8.3; lignin = 13:23; polyphenols = 0.26) have a relatively constant N release pattern on treatment with *P.corethrurus* inoculation. This is caused by the CM has a low energy content (Table 1). The decomposition of litter is depend on the combined activity of microbes and soil animals.The main contribution by the soil fauna is considered to be their comminution of detritus, which increases the available surface area and promotes microbial breakdown (Engelstad, 1991). Suthar(2007) reported that types of substrateor feeding material affected the biomass production of earthworm. Thus, it influenced the earthworm activity in composting the litter. Pashanasi (1992) also reported that the different organic matter types affected *P.corethrurus* growth. Itsignificantly affectedmineralization of nitrogen and microbial biomass accumulation which assisted N mineralization. Organic matters with a high C- organic can be a source of food and energy of earthworms. According to Martin and Lavelle (1992) geophagus earthworms such as *P.corethrurus* like substrate that contains a high C-organic for the C-assimilation process. vanVliet (2007) reported that the endogeicearthworm prefer food with a C/N ratio> 12.4. The highest assimilation rate occured in organic matters that are decomposed slowly. This results in line with reported by Tian*et al.,* (1997) which reported that the effect of earthworms on the decomposition and mineralization rate is more significant on the applications of low quality organic matters. Our results also indicate that earthworm inoculation can stimulate the overall net N mineralization of each type of organic matter applied into pot. Although a number of other studies have also suggested that earthworms can accelerate the mineralization of N from organic materials (Willems*et al.*, 1996; Bohlen *et al.*, 1997; Subler*et al.*, 1998; Helling and Larink, 1998), ours is one of the first studies to measure directly the influence of earthworms *P.corethrurus* on net mineralization in bulk soil through incubation experiment used the soil from sugarcane land with high inorganic input in long-term. The long-term purpose of this research is to find a sustainable sugarcane production system in Indonesia with a low external input by utilizing sugar agro-industry waste.

CONCLUSIONS

Based on this study clearly, inoculation of earthworm *P.corethrurus* can accelerate net ammonification, nitrification and N mineralization rates on various quality of organic matter from sugar agroindustry waste. It means that the earthworm inoculation reducing the net immobilization into microbial biomass and increase N availability in the soil.The highest N mineralization rate was found on the addition of mixture high and low quality organic matters (cattle manure+sugarcane trash and mixture cattle manure+filter cake). But the highest increase N mineralization occurred on the addition of sugar cane trash and mixture cattle manure+sugarcane trash when compared without earthworm inoculation. These results suggest that maintaining organic matter in the soil can increase earthworm activity in N mineralization process although the organic matters have a low quality.

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REFERENCES

Bisht R.H, PandeyD., Bhartiand KaushalB.R.(2003). Population dynamics of earthworms (Oligochaeta) in cultivated soils of central Himalayan tarai region Tropical Ecology Vol. 44, 2,229-234.

BityutskiiN.P.,SolovevaA.N, LukinaE.I., OleinikA.S.,ZavgorodnyayaY.A.,DeminV.V.and ByzovB.A. (2007). Stimulating effect of earthworm excreta on the mineralization of nitrogen compounds in soil. Eurasian Soil Science Vol.40, 4, 426-431.

BohlenP.J., Parmelee R.W., Edwards C.A. and McCartney D.M. (1997). Earthworm effects on carbon and nitrogen dynamics of surface litter in corn agroecosystems. Ecol. Appl. Vol7, 1341-1349.

DewiW.S., Yanuwiyadi B., Suprayogo D.and Hairiah K. (2006).Can agroforestrymaintainearthworm diversity after forest conversionto be agricultural land? Agivita Vol.28, 3, 198-220.

Engelstad F. (1991). Impact of earthworms on decomposition of garden refuse. Biol Fertil Soils Vol.12, 137-140

Fragoso C., BrownG.G., PatronJ.C., Blanchart E., Lavelle P., Pashanasi B., Senapati B., KumarT.(1997). Agricultural intensification, soil biodiversity and agroecosystem function in the tropics : the role of earthworm. Appl.Soil.Ecol. Vol. 6, 17–35.

HellingB.and LarinkO.(1998). Contribution of earthworms to nitrogen turnover in agricultural soils treated with different mineral N-fertilizers. Applied Soil Ecology Vol.9, 319-325.

Kale R.D. (1998).Earthworms nature's gift for utilization of organic waste. Pp.355-376. In :C.A.Edwards (ed) Earthworm Ecology. CRC.Press.LLC.Florida.

KaushalB.R., KandpalB., BishtS.P.S., BoraS., and DhapolaR. (1999).Abundance and seasonal activity of earthworms in croplands of Kumaon Himalayas. European Journal of Soil Biology Vol. 35, 171-176.

LamandeM.,HallaireaV.,CurmiaP., Pe´re`sG. and CluzeauD.(2003). Changes of pore morphology, infiltration andearthworm community in a loamy soil underdifferent agricultural managements.CatenaVol.54.637–649.

LeeK.E. (1985). Earthworms: Their Ecology and Relationships with soil and Land Use. Academic Press.

MartinA.and Lavelle P.(1992).Effect of soil organic matter quality on its assimilation by *millsoniaanomala,* a tropical geophagous earthworm.Soil Biology andBiochemistryVol. 24, 12, 1535-1538.

Nurhidayati, ArisoesilaningsihE., SuprayogoD., and HairiahK.(2011).Long-term impact of conventional soil management to earthworm diversity and density on sugarcane plantation in East Java. Journal of Nature Studies Vol.10,2,16-25

PashanasiB., Melendez G., SzottL., LavelleP.(1992). Effect of inoculation with the endogeic earthworm *Pontoscolexcorethrurus* (Glossoscolecidae) on N availability, soil microbial biomass and the growth of three tropical fruit tree seedlings in a pot experiment. Soil Biol.BiochemVol. 24,12, 1655-1659.

PérèsG., CluzeauG., CurmiD., and HallaireV.(1998).Earthworm activity and soil structure changesdue to organic enrichments in vineyard systems. BiolFertilSoils Vol.27,417–424.

Singh J. (1997). Habitat preferences of selected earthworm species and their efficiency in reduction of organic material. Soil Biology and Biochemistry Vol.29, 585-588.

SublerS., ParmeleeR.W., and AllenM.F.(1998).Earthworms and nitrogen mineralization in corn agroecosystems with different nutrient amendments. Applied Soil Ecology Vol. 9, 295-301

SutharS.(2007). influence of different food sources on growthand reproduction performance of compostingepigeics: *Eudriluseugeniae, Perionyxexcavatus*and P*erionyxsansibaricus*. *Applied ecology and environmental research* Vol.5,2, 79-92.

TianG.,Kang B.T., Brussaard L., and SwiftM.J.(1997).Soil fauna mediated decomposition of plant residues under constrained environmental and residue quality and conditions. In: Cadish, G and Giller, K.E (Eds). Driven by nature: Plant litter quality and decomposition. CAB International Wallingford. UK.pp.125-134.

vanVlietP.C.J., van der Stelt B.,Rietberg P.I.and de Goede R.G.M.(2007). Effects of organic matter content on earthworms and nitrogen mineralization in grassland soils European Journal of Soil Biology Vol.43, S222-S229.

WillemsJ.J.G.M., MarinissenJ.C.Y.and Blair J. (1996).Effects of earthworms on nitrogen mineralization. Biol Fertil Soils Vol.23, 57-63