INFLUENCE OF NEMATODE-BACTERIAL INTERACTIONS ON N AND P MINERALISATION IN SOIL AND ON DECOMPOSITION OF CROP RESIDUES DURING AEROBIC COMPOSTING

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Abstract. The chemical composition of the crop residues such as the N and lignin content is one of the important factors that influence microbial activity, including their efficiency and contribution to the decomposition process. The objective of the present study was to compare the effects of organic substrates of different carbon to nitrogen (C:N) ratios viz; cabbage (*Brassica oleracea*) leaves, Soybean (*Glycine max*) stover and paddy (*Oryza sativa*) straw on population densities of a bacterivorous nematode, *Cephalobus persegnis* and its effect on N and P mineralization in soil microcosms and rate of organic matter decomposition in aerobic composting. The bacterivorous nematode, *C. persegnis* enhanced the release of ammonium nitrogen (NH_4^+-N) in presence of soybean (*Glycine max*) stover and paddy (*Oryza sativa*) straw in soil microcosms. Sampling at 15-day intervals revealed a progressive increase in the population densities of *C. persegnis* up to 45 days, followed by a decline. The bacterial densities in soil were significantly low in presence of nematodes on all days of sampling indicating the grazing effect of the nematodes. The microbial biomass carbon (MBC) was significantly higher in the presence of nematodes, on all days of sampling. A second trial on the rate of decomposition of the above crop residues during aerobic composting revealed enhanced reduction in C:N ratios in presence of nematodes on 75 and 90 days of incubation, in paddy straw treatments.
Keywords: *Cephalobus persegnis, carbon-to-nitrogen ratio, mineralisation, cabbage, paddy, soybean*

Introduction

The crop residues are incorporated into the soil as a soil amendment or used as composting material. The chemical composition (carbon-to-nitrogen ratio) of the crop residue and their rate of decomposition in soil is influenced by soil moisture, temperature and microbial interactions. The biotic and abiotic interactions resulting in nutrient dynamics in the soil also influence the rate of decomposition of organic matter in the soil. Such interactions are poorly understood as limited investigations have been carried out on this aspect (Alphei et al., 1996; Schutter and Dick, 2001; Chigineva et al., 2009; Carrillo et al., 2011; Ball et al., 2014). Among the biotic interactions, bacterivorous nematodes are an important component of the soil fauna and it needs to be established if they cause a significant effect in enhancing the rate of nutrient mineralization in the soil and accelerating the process of organic matter decomposition.

The carbon-to-nitrogen (C:N) ratios in organic matter incorporated in soil are variable and range from 20:1-30:1 in legumes and farm yard manure to as high as 100:1 in certain cereal straw residues. Incorporating organic matter of wide C:N ratio (more than 50:1) into the soil under favourable conditions for decomposition results in an increase in heterotrophic microbial populations with production of

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large amount of CO_2 (Das, 2008). Incorporation of wheat straw is reported to increase CO_2 fluxes from 0.30 to 1.30 kg CO_2 $ha^{-1}d^{-1}$ (Curtin et al., 1998). When carbon is abundant, an increase in microbial biomass may result in immobilisation of N. Under such circumstances, mineralization may be increased by organisms such as bacterivorous nematodes that graze on soil bacteria and enhance soil nutrient availability (Ferris et al., 1997).

Bacterivorous nematodes constitute 20-50% of the total number of nematodes present in soil. Sometimes their proportion reaches 90-99% at sites of high microbial activity (Griffiths, 1989). Their interactions with microbes in the soil resulting in enhanced nutrient release have been reported by several workers (Coleman et al., 1984; Freckman, 1988; Griffiths, 1994).

Bacterivorous nematode, *Cephalobus persegnis* Bastian 1965, was found to be one of the most abundant rhabditid in the Indo-gangetic rice-wheat growing regions of India (Singh, 2007). The basal threshold temperature of this nematode is 4.2 °C and the upper threshold is 42.2 °C with an optima of 32.2 °C (Venette and Ferris, 1997). This thermal adaptation is possibly responsible for the predominance of this species in the soil. Further, *C. persegnis* is reported to enhance the release of NH_4^+–N in soil microcosms (Kamra et al., 2003).

The present study was undertaken to compare the effects of three crop residues with different C:N ratios, viz cabbage (*Brassica oleracea* L.) leaves, soybean (*Glycine max* L.) stover and paddy (*Oryza sativa* L.) straw on population densities of *C. persegnis* and its effect on N and P mineralization in soil microcosms for a period of 75 days. In a second trial, the rate of decomposition of the above three substrates during aerobic composting was investigated in the presence and absence of the bacterivorous nematode for the period of 90 days.

Material and methods

Isolation of nematodes

The soil samples for isolation of nematodes were collected row-wise in a zig zag pattern, using an auger, from the upper 30 cm layer of soil in a field under rice-wheat cropping system, located at the Indian Agriculture Research Institute (IARI), New Delhi, India. The composite samples were prepared by collecting 50 samples of 500 cm^3 soil, from 1 hectare field area. The nematodes were extracted using ten samples, each of 500 cm^3 soil, by Cobb's decanting and sieving technique (Cobb, 1918) followed by modified Baermann funnel technique (Schindler, 1961). The nematode suspension collected after 48 h was examined for presence of the dominant bacterivorous nematode. A single gravid female of the predominant bacterivorous nematode, *C. persegnis*, was handpicked and placed on water agar plate supplemented with bacteria isolated from the same soil samples. After egg laying, the juveniles were allowed to develop and multiply on the plates for 4 weeks at 32 °C. Thereafter the nematodes were extracted from the plates by Schindler's method. Nematodes so collected were distributed on fresh agar plates. This process was repeated for mass multiplication of the nematodes. They were processed by the Seinhorst method (Seinhorst, 1959) and identified at the Division of Nematology, IARI, New Delhi, India. The gnotobiotic culture plates of the nematode were maintained in a BOD incubator at 32 ± 2 °C for experimental trials.

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Isolation and identification of bacteria

The soil samples used in the isolation of nematodes were also used for the isolation of bacteria in order to retain the native soil bacteria. Colony forming units (CFU's) of bacteria were estimated using 10 cm^3 of air dried soil. The soil was thoroughly vortexed using 100 cm^3 of sterile distilled water for 5 minutes. One mL of the aliquot was drawn from this suspension to make a serial dilutions upto 10^{-16} of which 10 microlitre was plated on nutrient agar plates (Wollum, 1982). Plates were incubated at 25 ± 1 °C for 24 h and colonies were identified under a stereoscopic microscope at 6.7× based on colony morphology and standard biochemical tests (Benson, 2002). Colonies developed on the plates belonged to the genera *Bacillus*, *Pseudomonas,* and *Enterococcus*.

Soil and substrate Sterilisation

The sandy loam soil from the field of IARI was used for all the experiments. The soil was steam sterilized at 120^oC, 103421.35 Pa pressure for 1 h. Three random samples of sterilized soil were processed by Cobb's decanting and sieving technique (Cobb, 1918) followed by modified Baermann funnel technique (Schindler, 1961) and examined to ensure that the sterilized soil was free from nematodes. The substrates were chopped and put in autoclavable bags for steam sterilization at 120^oC, 103421.35 Pa pressure for 30 min. each. This sterilized soil and crop residue substrates were used for preparing microcosms.

Experiment 1: Nitrogen and phosphorus mineralization in soil

The experiments were conducted in soil microcosms using 150 g sterilised soil in 200 cm^3 plastic cups inoculated with sterilised substrates, bacteria and nematodes and incubated for a period of 75 days. All the three substrates, viz cabbage leaves (C:N ratio: 30:1), soybean stover (C:N ratio: 56:1) and paddy straw (C:N ratio: 90:1) were shredded to about 1 cm size and mixed thoroughly with sterilized soil at the rate of 1 g residue carbon (C) per 100 cm^3 soil before filling in microcosms. The microcosms were inoculated with freshly prepared bacterial suspension at the rate of 1.69×10^{16} CFUs g^{-1} soil and kept for two days at 32 ± 2 °C for 48 h for the bacteria to establish, prior to the nematode inoculation (5 g^{-1} soil). The treatments thus comprised of each of the three substrates with and without nematodes, and respective no-substrate controls. For each treatment, four replications were maintained. The moisture was adjusted to field capacity and maintained by replacing the weight loss with distilled water at 5 day intervals during incubation period. Four sets of microcosms were established to allow destructive sampling at 15 day intervals for determination of nematode and bacterial densities, microbial biomass carbon (MBC), ammoniacal N (NH_4^+–N), nitrate N (NO_3^-–N) and available phosphorus (P) The nematode population density was estimated after extraction from 90 g soil by Cobb's decanting and sieving technique (Cobb, 1918) followed by modified Baermann funnel technique (Schindler, 1961) and CFU's of bacteria were estimated using 1 g soil on nutrient agar plates by serial dilution method (Wollum, 1982). The MBC was estimated by fumigation extraction method (Voroney et al., 1993), and NH_4^+–N and NO_3^-–N were analysed by Kjeldahl distillation method by adding MgO and Devarda alloy,

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respectively (Keeney and Nelson, 1982). The available phosphorus (P) was estimated by Olsen's method (Olsen, et al., 1954).

Experiment 2: Organic matter decomposition in aerobic composting

Plastic trays (45×35×15 cm), containing organic substrates (cabbage leaves, paddy straw and soybean stover), denematised cow dung, soil and composting starter were mixed in the ratio of 8:1.0:0.5:0.5 on weight basis. Finally, each tray contained 800 g substrate, 100 g cow dung, 50 g soil and 50 g composting starter. The treatments comprised of each of the three substrates or no substrate with and without nematodes. In the treatments with no substrate, 800 g soil is used instead of the 800 g substrate. Each treatment was replicated four times. The inoculation of bacteria and *C. persegnis* were carried out as in the experiment described above. The trays were incubated at 32 ± 2 °C for 90 days. The nematode population densities were estimated after extraction from 60 g composting material by Cobb's decanting and sieving technique (Cobb, 1918) followed by modified Baermann funnel technique (Schindler, 1961), the total carbon and total N was estimated by wet oxidation (Snyder and Trofymow, 1984) and Kjeldahl distillation (Bremner, 1965), respectively, at 15 days interval.

Statistical analysis

The data on various parameters i.e., nematode, bacterial counts, NH_4^+–N, NO_3^-–N, available phosphorus (P) and microbial biomass carbon (MBC) were analysed using 'analysis of variance' (ANOVA) technique (Gomez and Gomez, 1984) using DSAASTAT, version, 1.1 statistical package (Onofri, 2007) available at http://www.unipg.it/~onofri/DSAASTAT/DSAASTAT.htm. The data on nematode population were square root transformed prior to analysis to meet the assumptions of ANOVA and conclusion drawn in the transformed scale. However, only untransformed arithmetic means of all data are presented. The differences at $P < 0.01$ and $P < 0.05$ level were considered statistically significant using the least significant difference (LSD) test.

Results and discussion

Experiment 1

The population densities of *C. presegnis* increased significantly up to 30 days of incubation in soybean stover treatment, while in cabbage and paddy straw treatments, an increase was observed up to 45 days followed by a progressive decline in both (*Fig. 1*). The bacterial densities were significantly low in the presence of the nematode than in their absence, in all three substrate treatments, on days 15, 30 and 45, indicating the grazing effect of the nematode (*Fig. 2*). Such a decline in bacterial densities due to grazing effect of bacterivorous nematodes has been reported by several other workers (Gould et al., 1981; Anderson et al., 1983). However, a decline in bacterial CFUs does not necessarily indicate a reduced bacterial activity (Djigal et al., 2004).

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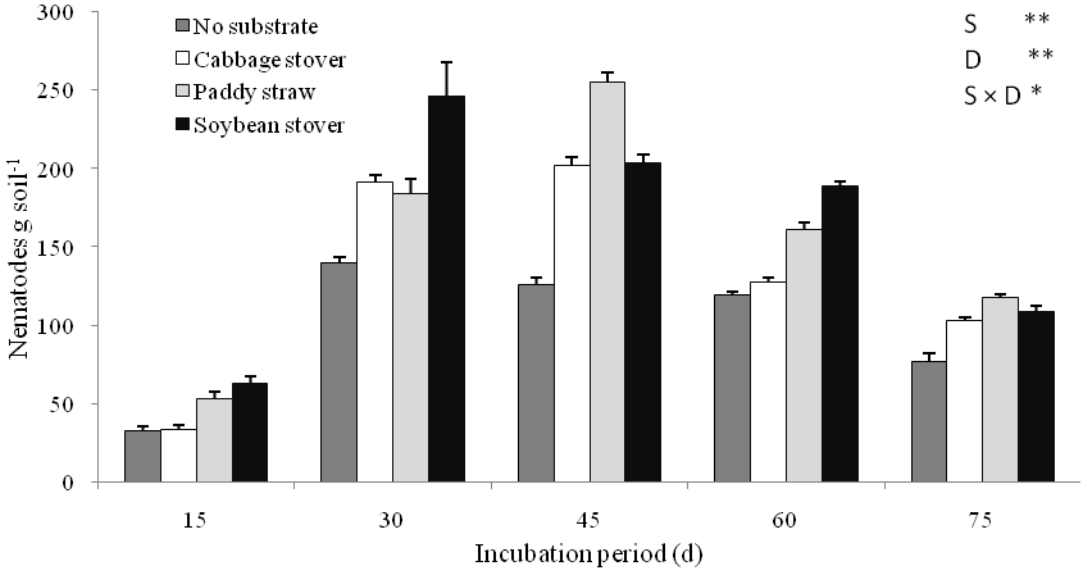
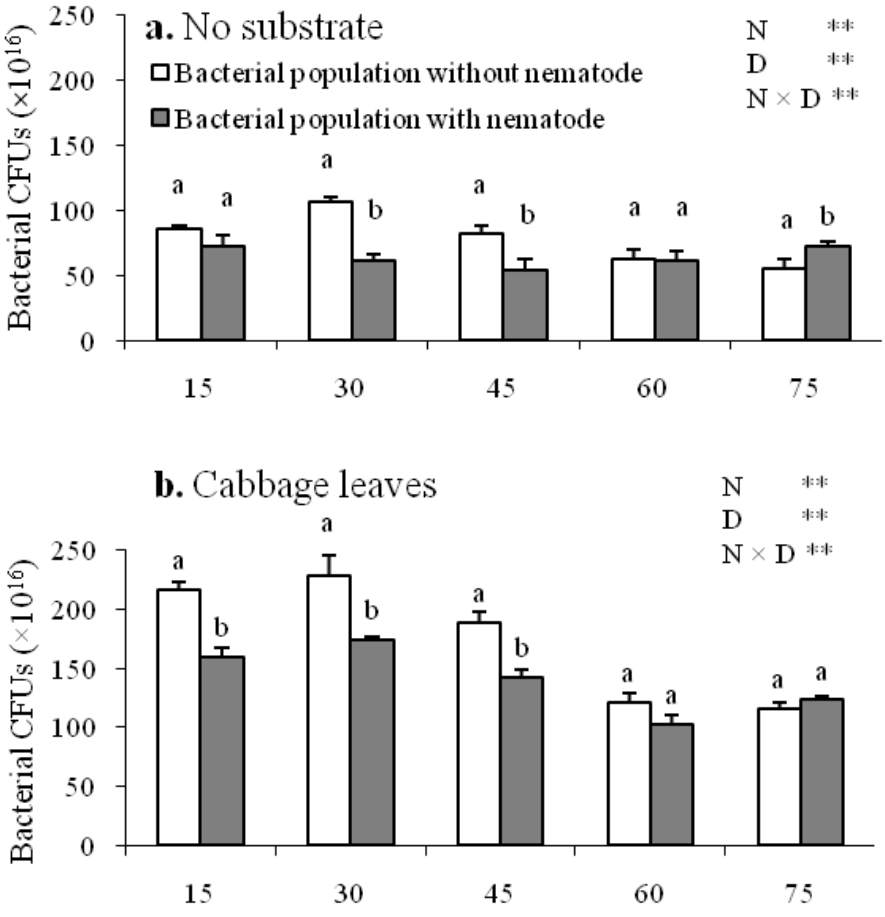


Figure. 1. *Population density of Cephalobus persegnis across different substrates over 75 days of incubation period during N and P mineralisation. Bars represent standard errors. Significance of the factors and their interactions (S = substrate, D = days, S x D = the interaction) are shown as * and ** which denote P < 0.05 and <0.001 respectively.*



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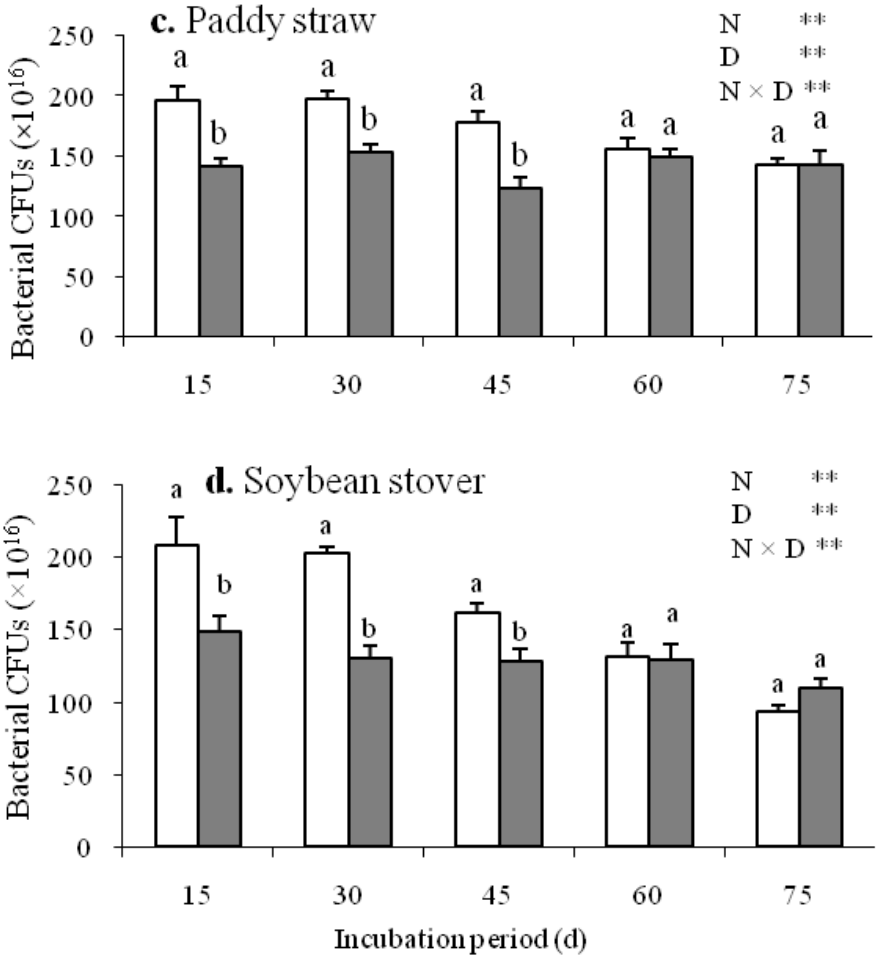

Figure. 2. *Bacterial population densities in presence and absence of Cephalobus persegnis across different substrates (Figure. 2a-d) over 75 days of incubation period. Bars represent standard errors. Different latter over pair of bars indicate a significant (P < 0.05) nematodes effect for that sampling period and substrate. Significance of the factors and their interactions (N = nematodes, D = days, N x D = the interaction) are shown for each plate as *, ** and ns, which denote P < 0.05, <0.001and non-significant respectively.*

The changes in nematode and bacterial densities were reflected in total microbial biomass carbon (MBC) on respective days of sampling (*Fig. 3*). The levels were significantly higher in presence of *C. persegnis*, than in its absence in all 3 substrates, on all days of observations, except day 15 in paddy straw treatments (*Fig. 3*). The progressive increase in MBC for 30-45 days of incubation, followed by a decline on days 60 and 75 were commensurate with the respective population densities of *C. persegnis* and bacteria (*Fig.1 and 2*). In the absence of organic substrate, this trend was not observed, rather a progressive decline was observed from 800 µg C g^{-1} dry soil to 400 µg C g^{-1} dry soil, from day 15 to day 75, indicating lowering microbial activity and mineralisation. The microbial biomass of carbon (MBC) constitutes labile carbon that declines faster and is restored faster than the non-labile carbon and is therefore a more sensitive indicator of carbon dynamics in agroecosystems (Blair et

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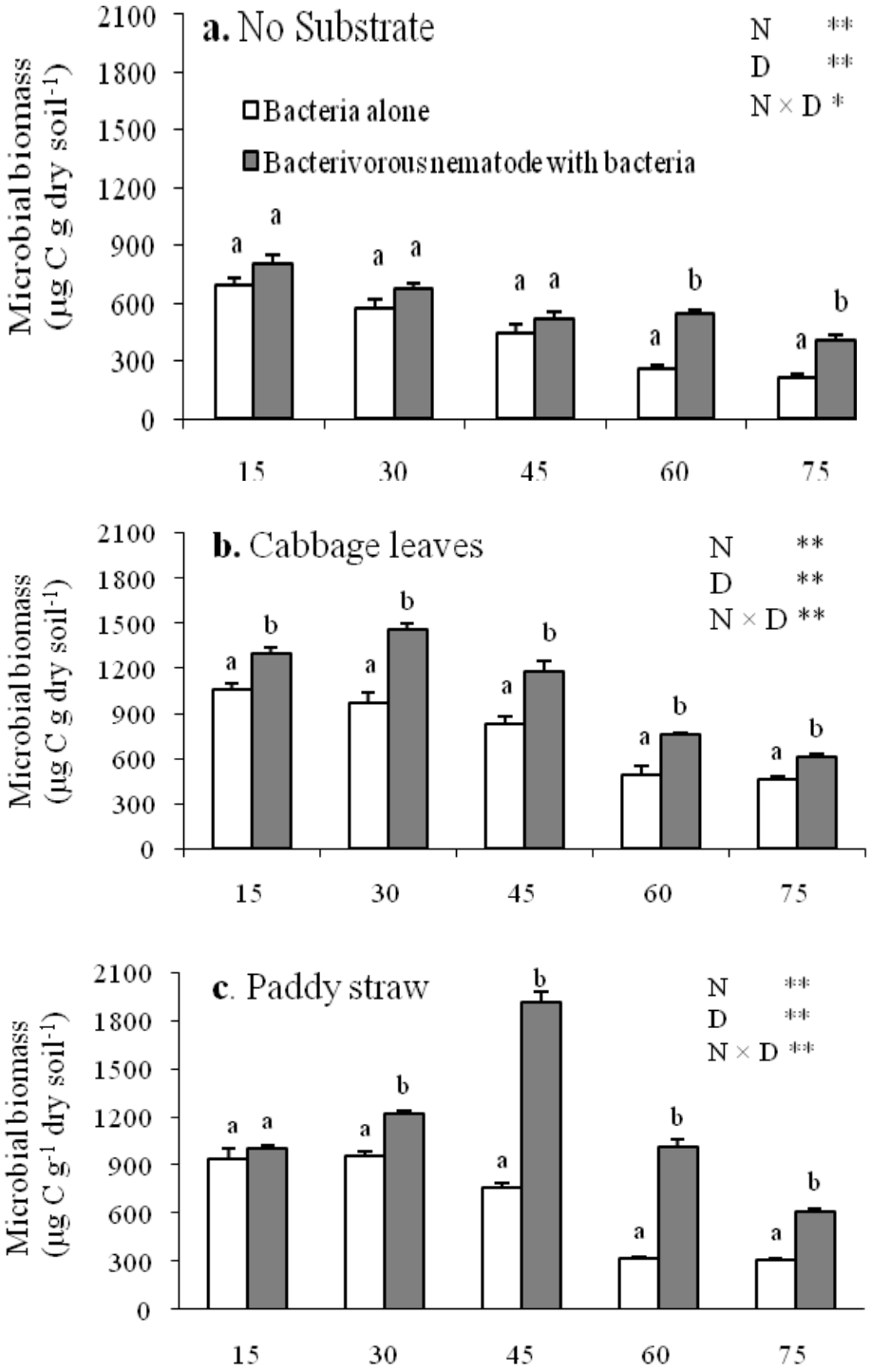
al., 1995). There are microbes which do not proliferate on the synthetic media, but account for the MBC and contribute towards the process of nutrient mineralization.



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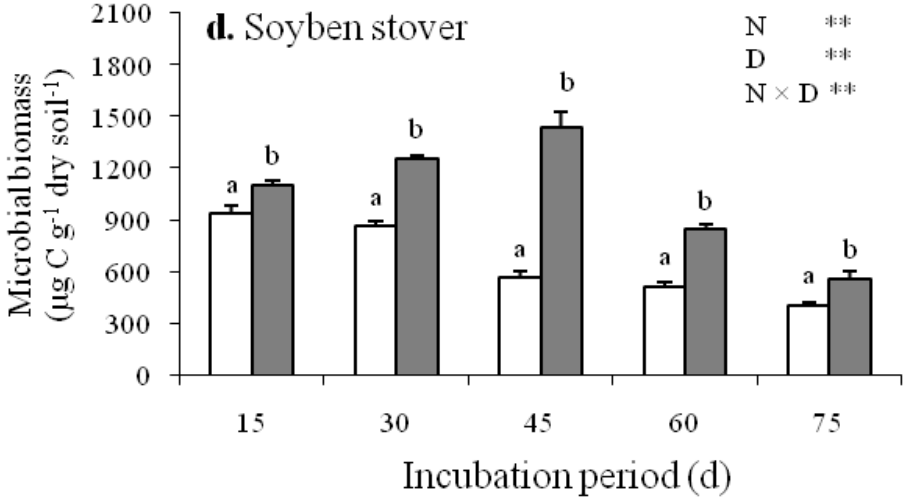
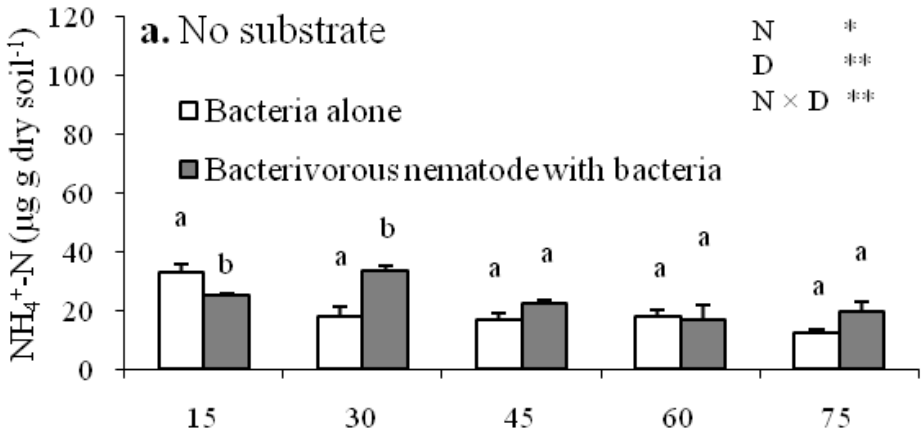


Figure. 3. *Influence of bacteria alone or Cephalobus persegnis plus bacteria on microbial biomass carbon across different substrates (Figure. 3a-d) over 75 days of incubation period. Bars represent standard errors. Different latter over pair of bars indicate a significant ($P < 0.05$) nematodes effect for that sampling period and substrate. See Figure 2 legend for the details on design and statistical results.*

The presence of *C. persegnis* resulted in significantly enhanced levels of NH_4^+–N in paddy straw treatments on days 15, 30 and 45 while in soybean stover treatment, these levels were significantly higher on all days of observation, except day 45 (*Fig. 4*). The trend that was noteworthy was the progressive increase in NH_4^+–N levels up to 45 days, followed by a decline on days 60 and 75 in the above two treatments, commensurate with the population density pattern of *C. persegnis* observed during that period. This supported the hypothesis that nematodes excrete inorganic nitrogen, mainly as NH4 (Ferris et al., 1998) and stimulate nitrogen mineralisation through their grazing activity. Increase in nitrogen mineralisation in the presence of bacterial-feeding nematodes has been reported in many studies (Ingham et al., 1985; Ferris et al., 1998; Lokupitiya et al., 2000; Kamra et al., 2003).



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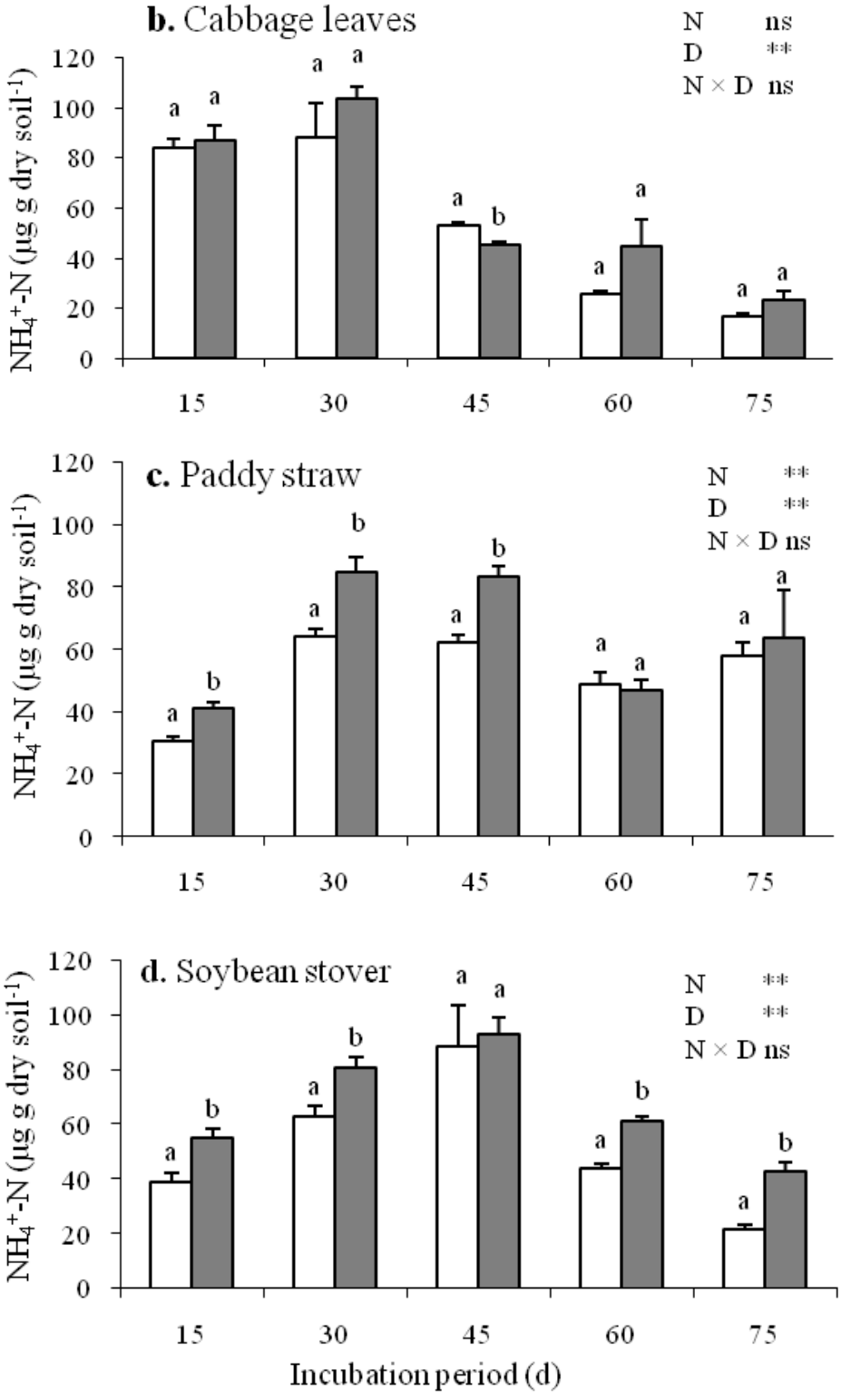

Figure. 4. *Influence of bacteria alone or Cephalobus persegnis plus bacteria on release of ammonical nitrogen (NH_4^+–N) across different substrates (Figure. 4a-d) over 75 days of incubation period. Bars represent standard errors. Different latter over pair of bars indicate a significant ($P < 0.05$) nematodes effect for that sampling period and substrate. See Figure 2 legend for the details on design and statistical results.*

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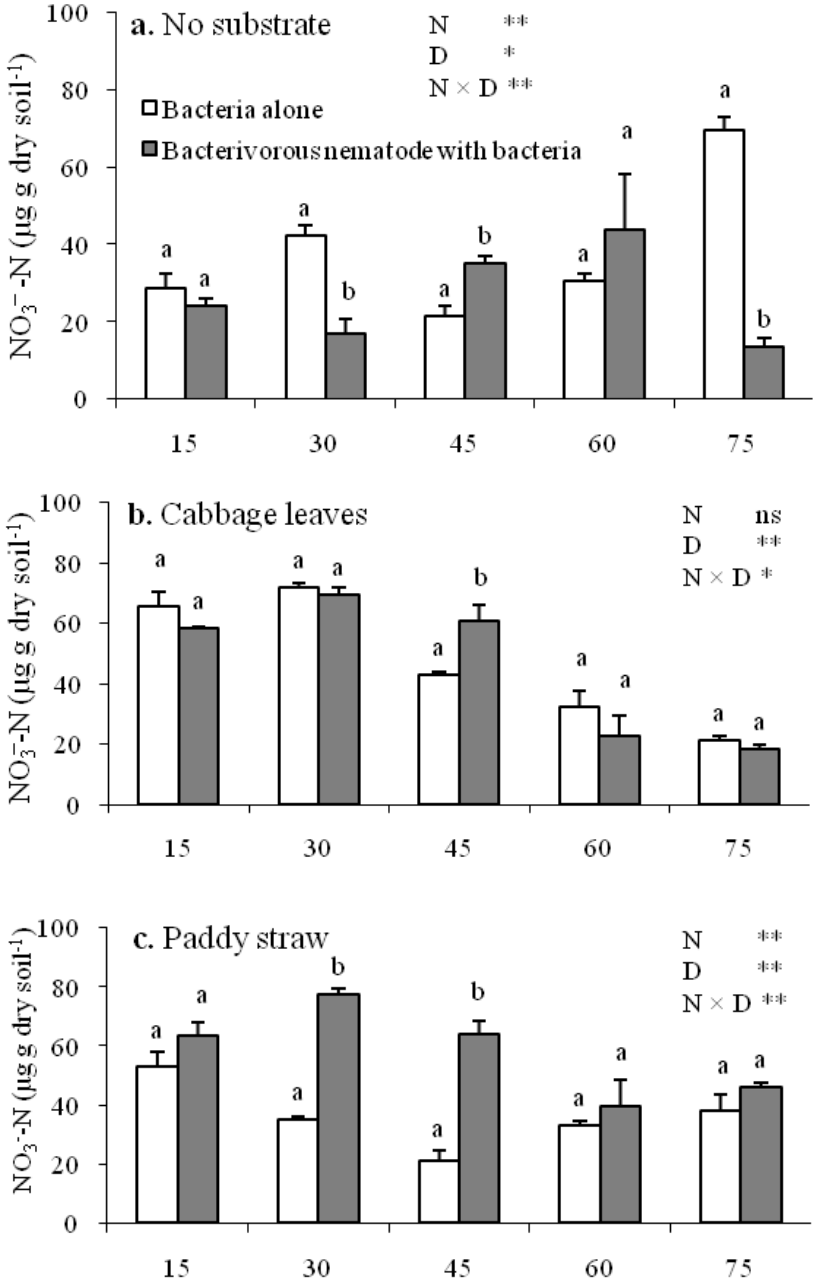
The NO_3^-–N levels showed an enhanced release in the presence of *C. persegnis* on days 30 and 45 in paddy straw treatments and day 45 in cabbage treatment (*Fig. 5*). Increase in nitrification in the presence of single species of bacterivorous nematode or the presence of nematode communities was also demonstrated by Bouwman et al., (1994) and Xiao et al., (2010). Gebremikael et al. (2014) also observed consistently higher concentration of NO_3^-–N in presence of nematodes than in their absence. However, release of NO_3^-–N in soyabean stover treatments is consistently less in presence of nematodes compared to their absence. In the absence of substrates, level NO_3^-–N is significantly lower on days 30 and 75. The inconsistent effects were possibly due to low levels of nitrifying bacteria and needs to be investigated.



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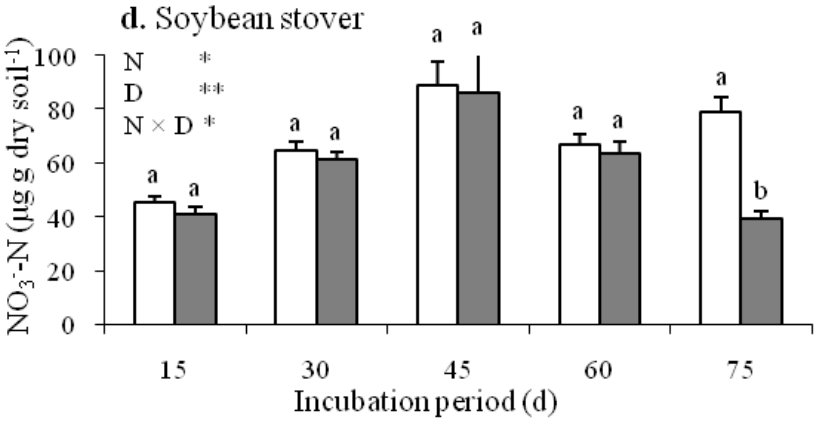
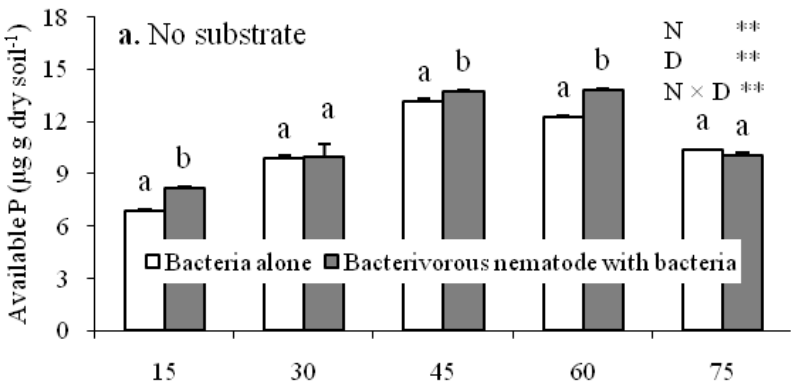


Figure. 5. *Influence of bacteria alone or Cephalobus persegnis plus bacteria on release of nitrate nitrogen (NO_3^-–N) across different substrates (Figure. 5a-d) over 75 days of incubation period. Bars represent standard errors. Different latter over pair of bars indicate a significant (P < 0.05) nematodes effect for that sampling period and substrate. See Figure 2 legend for the details on design and statistical results.*

The enhanced levels of P in presence of *C. persegnis* were observed on all days of sampling except day 45 in soybean stover, on days 45 and 60 in paddy straw treatment and days 15, 60 and 75 in cabbage treatment (*Fig. 6*). Therefore, a consistent relationship of enhanced P mineralisation in presence of *C. persegnis* could not be confirmed. Few studies have been made on the N and P content of nematodes. The P content is reported to be between 0.1--0.6 % biomass of nematodes (Dropkin and King, 1956; Hunt et al., 1987). The assimilation efficiencies are between 30--60%; thus, it is expected that nematodes would release P after feeding on bacteria. Enhanced levels of P have been reported in presence of bacterivorous nematode, *Mesodiplogaster* sp. by Coleman et al. (1977) and Anderson et al. (1981) in microcosms experiments. Coleman et al. (1977) found enhanced P mineralisation in microcosms with sterilised soil inoculated with bacteria alone or bacteria in combination with bacteriophagous amoebae or nematodes. In each case, mineralisation was greater in presence of the animal. However, Cole et al. (1978) and Woods et al. (1982) reported no enhanced P mineralisation in presence of bacterivorous nematodes.



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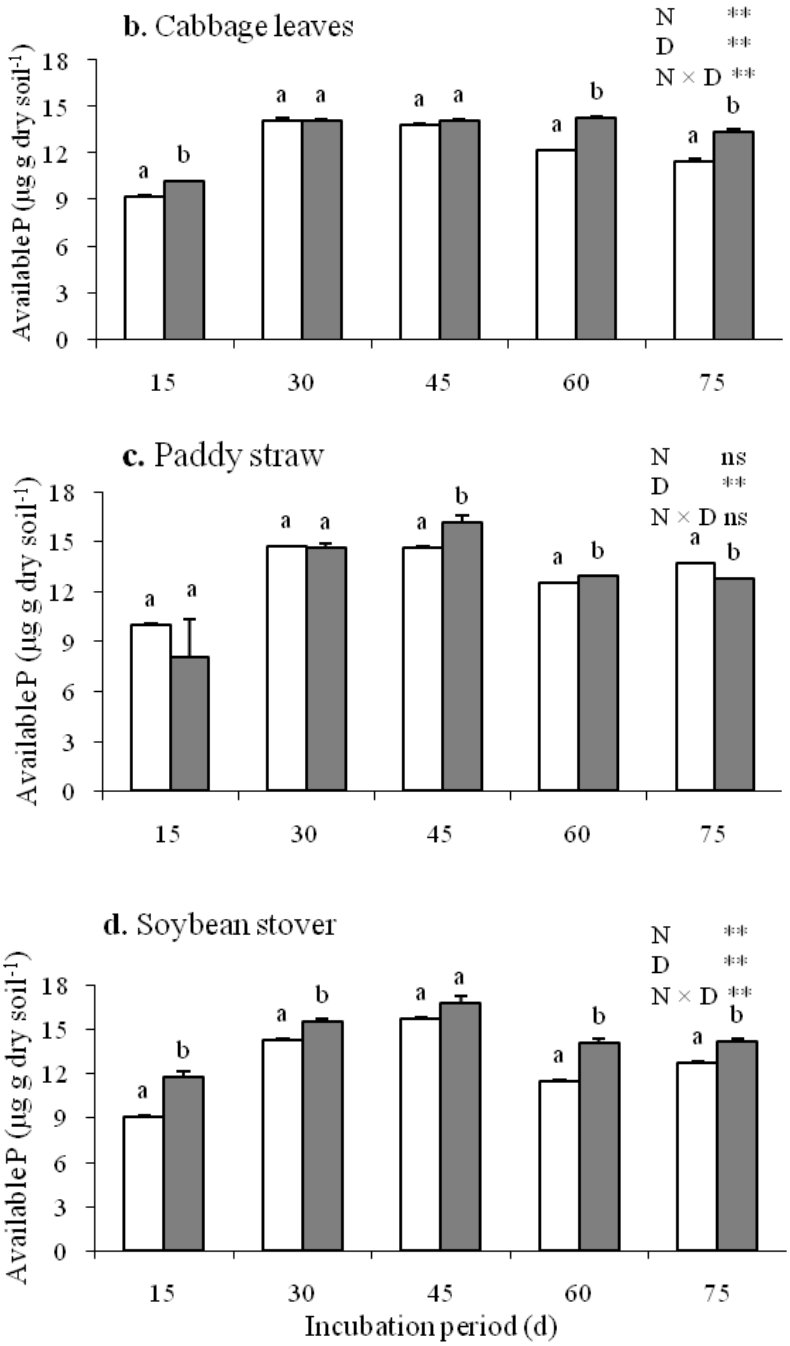

Figure. 6. *Influence of bacteria alone or Cephalobus persegnis plus bacteria on release of phosphorus across different substrates (Figure. 6a-d) over 75 days of incubation period. Bars represent standard errors. Different latter over pair of bars indicate a significant (P < 0.05) nematodes effect for that sampling period and substrate. See Figure 2 legend for the details on design and statistical results.*

Experiment 2

This experiment was conducted to determine the effect of bacterivorous nematode on rate of composting ie., if besides enhancing the rate of nutrient mineralisation,

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these nematodes could enhance the rate of composting, ie the time at which C:N ratio becomes constant (indicating no further decomposition) in the composting substrates.

The population densities of bacterivorous nematodes during aerobic composting showed similar trend in cabbage and soybean stover compost with respect to period of incubation. However, the nematodes were much higher on days 30, 45 and 60 in soybean stover compared to other substrates. In paddy straw composting, the densities of bacterivorous nematodes increased progressively up to 45 days followed by a progressive decline on days 60, 75 and 90. Similar trend was observed in the absence of organic substrate; although the density of bacterivorous nematodes was much lower (*Fig. 7*). As is evident from *Fig.8*, the decline in C:N ratios in various substrates was not affected significantly by presence of bacterivorous nematodes in cabbage or soybean composting on any day of sampling. It has been found that, in nutrient rich conditions (amended soil), the presence of nematodes may not significantly enhance organic matter decomposion and the subsequent nutrient mineralization (Ingham et al., 1985; Bjornlund et al., 2012; Gebremikael et al., 2015). Gebremikael et al (2015) found that there is no significant contribution of entire free living nematode community to C mineralisation either in native soil organic matter or added organic matter (grass covered amendments). However, this was attributed to lower nematode density in the experiment or significant decrease in nematode population density over the time. In paddy compost, the decline in C:N ratio due to presence of bacterivorous nematodes was found to be significant on days 75 and 90. This was supported by the relatively high number of bacterivorous nematodes (700 g^{-1} compost) maintained in paddy compost compared to a low number in cabbage (about 100g^{-1} compost) and soybean (250g^{-1} compost). There is a need to identify and quantify the microbial community present in each of these substrates to further understand and interpret the biotic interactions during composting.

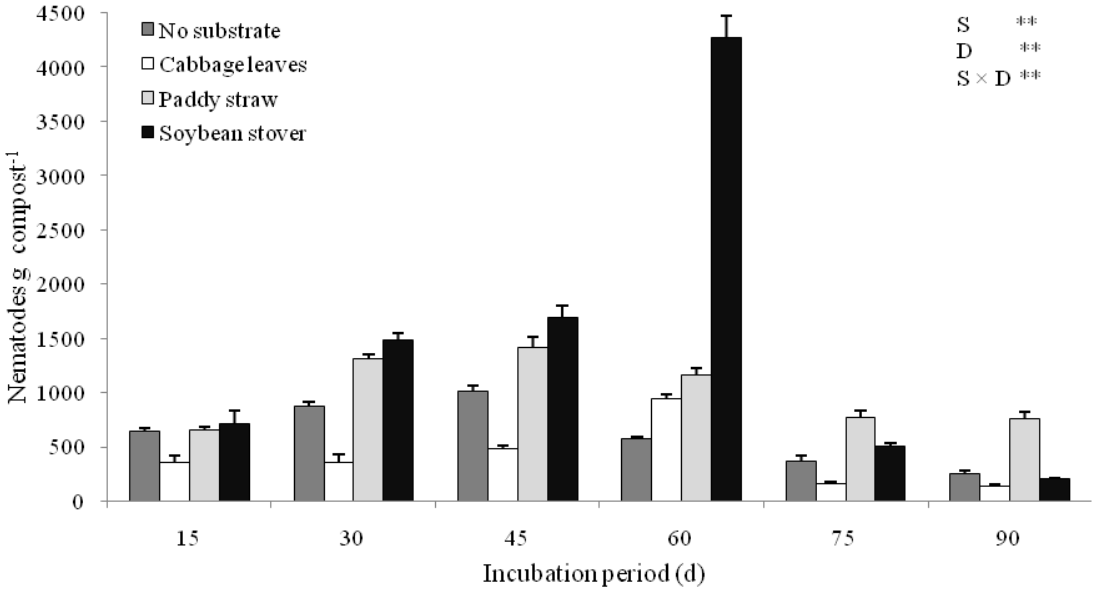

Figure. 7. *Population density of Cephalobus persegnis across different substrates over 90 days of incubation period during aerobic composting. Bars represent standard errors. Significance of the factors and their interactions (S = substrate, D = days, S x D = the interaction) are shown as ** which denote P < 0.01.*

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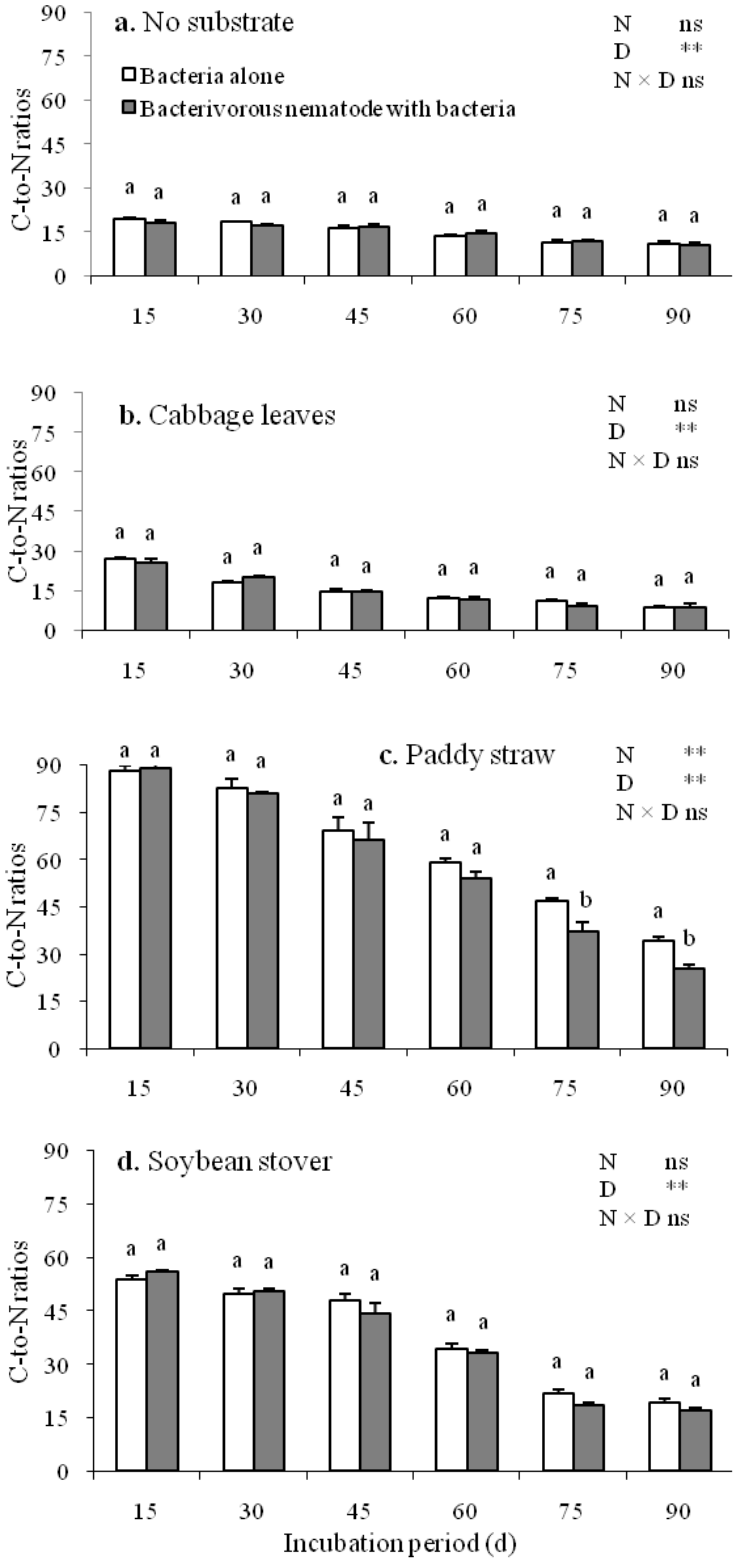

Figure 8. *Influence of bacteria alone or Cephalobus persegnis plus bacteria on C:N ratios across different substrates (Figure. 8a-d) over 90 days during aerobic composting. Bars represent standard errors. Different latter over pair of bars indicate a significant ($P < 0.05$) nematodes effect for that sampling period and substrate. See Figure 2 legend for the details on design and statistical results.*

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Bacterivorous nematodes can hasten the process of decomposition, as observed for a recalcitrant substrate like paddy straw. Compost can serve as an efficient delivery system for beneficial bacterivorous nematodes in soil as it can help in their establishment and also enhance the rate of mineralisation in soil, in presence of organic matter to achieve desirable C:N ratio (25-30:1). However, this hypothesis needs to be tested under field conditions.

Our results contribute to the evidence that the densities and activity of bacterivorous nematodes and bacteria present in the soil are influenced by the composition of the organic substrates. The presence of *C. persegnis,* significantly increased the microbial biomass carbon (MBC) and NH_4^+–N in a soil in presence of organic substrates like soybean stover or paddy straw. The nematode could also enhance the rate of decomposition of paddy straw during aerobic composting although the reduction in C:N ratios was significantly different from treatments without nematodes only on days 75 and 90 of incubation. However, in the present study contribution of single species of bacterivorous nematodes to nutrient mineralisation and organic matter decomposition was studied in the controlled environment but in realistic condition, decomposition process is regulated by several biotic and abiotic factors. Hence as suggested by Gebremikael et al (2015) studies are needed for realistic determination of contribution of entire free-living nematode communities including all feeding groups and indigenous microbial communities as occur in nature and management changes that are required for increasing the availability of nitrogen and other minerals especially in organic and low input farming systems.

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