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2 **Nitrogen cycling and Relationships between Ammonia Oxidizers and Denitrifiers**
3 **in a Clay-Loam Soil**

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1 **Abstract**

2 This study investigated the effect of municipal solid waste (MSW) compost (0, 50,
3 and 100 t/ha) on N cycling and the microorganisms involved in it, in a clay-loam soil.
4 After a release of nitrates (NO_3^- -N) in the first 6 days after compost incorporation, soil
5 NO_3^- -N content remained constant in all the treatments by day 62 suggesting
6 immobilization of N. Then, soil NO_3^- -N content increased, especially in the highest
7 compost dose implying that the immobilization effect has been relieved, at least to
8 some extent. *amoA* gene copies of ammonia oxidizing archaea (AOA) and ammonia
9 oxidizing bacteria (AOB) followed strictly the pattern of soil NO_3^- -N content
10 throughout the study providing evidence that both groups were involved in ammonia
11 oxidation and changes in their population can be used as ‘indicator’ for predicting
12 changes in soil nitrification status. Moreover, the strong correlation between AOA
13 and AOB *amoA* copies (R^2 : 0.94) and the high slope (13) of the curve suggest that
14 AOA had probably a more important role on ammonia oxidation under conditions of
15 low ammonia availability. Denitrifying genes (*nirS*, *nirK*, *nosZ*) also followed the
16 general pattern of soil NO_3^- -N and they were strongly correlated with both groups of
17 ammonia oxidizers, and particularly archaea, suggesting strong interrelationships
18 among them. Losses of N through denitrification, as they were estimated from total
19 nitrogen decrease, were inversely related to soil NO_3^- -N content. Similar to ammonia
20 oxidizers, denitrifying gene copies did not differ among compost treatments an effect
21 that could be probably explained by the low availability of organic-C of the MSW
22 compost and hence the competition with aerobic heterotrophs.

23 **Keywords:** ammonia oxidizing archaea; ammonia oxidizing bacteria; nitrification;
24 immobilization

1 **Introduction**

2 Application of organic amendments to agricultural lands restores soil organic matter
3 (SOM), improves soil structure, stimulates microbial activity, and supplies crops with
4 essential nutrients (García-Gil et al. 2000; Hargreaves et al. 2008). In order to achieve
5 sustainable management of soils, to maximize benefits to crops, and to eliminate
6 environmental impacts, detailed knowledge of C and N turnover contained in the
7 organic amendments is needed. Such information will enable us to estimate the
8 compost dose, the timing of application, and the interval of application to effectively
9 couple nutrient release with crop nutrient requirements.

10 Nitrogen mineralization rates of various organic substrate vary greatly, with those
11 from animal sources demonstrating the greatest rates (Busby et al. 2007; Cordovil et
12 al. 2005). In general, municipal solid waste (MSW) composts are characterized by
13 low rates of N release (Mkhabela and Warman 2005) as well as by immobilization
14 phenomena (Busby et al. 2007; García-Gil et al. 2000) which may inhibit growth and
15 decrease yields (Hargreaves et al. 2008). Availability of N (Madrid et al. 2011), the
16 C/N ratio of the substrate (Tognetti et al. 2008) soil properties and environmental
17 conditions (Kim et al. 2011) have been identified as the most critical parameters
18 controlling N mineralization, its subsequent transformation and eventually its
19 availability to crops.

20 To date, limited information is available for the microorganisms involved in N
21 cycling in compost-amended soils, particularly for ammonia oxidizers and
22 denitrifiers, their interrelationships, and the environmental factors affecting their
23 abundance and activity. Since the discovery of archaeal species encoding for *amoA*
24 gene (Venter et al. 2004) a substantial effort has been directed towards the
25 understanding of their role in ammonia oxidation (Könneke et al. 2005; Lehtovirta-

1 Morley et al. 2011; Tourna et al. 2011). Existing knowledge suggests that soil pH, and
2 particularly acidic soils, (Gubry-Rangin et al. 2010; Zhang et al. 2012) and NH_4^+ -N
3 availability (Höfferle et al. 2010; Levicnik-Hofferle et al. 2012; Verhamme et al.
4 2011) regulate the growth and activity of ammonia oxidizing archaea (AOA).
5 Oxidation of ammonia released from soil organic matter (SOM) or added organic-N
6 was accompanied by increases in abundance of the AOA *amoA* gene (Levicnik-
7 Hofferle et al. 2012). AOA also appeared to dominate ammonia oxidation in an acidic
8 peat soil which was released through the mineralization native organic matter
9 (Stopnisek et al. 2010). Similarly, application of biosolids at low rates (27 t/ha)
10 induced the growth of AOA only while higher applications favored the growth of both
11 AOA and ammonia oxidizing bacteria (AOB) (Kelly et al. 2011). In addition AOA
12 were found to be the dominant microbes throughout the composting of cattle manure
13 suggesting that they may play a role in ammonia oxidation during composting and
14 probably during the incorporation of compost to the soil (Yamamoto et al. 2011).
15 Another important issue is the losses of N through denitrification. Overall, few studies
16 have dealt with the effect of incorporation of organic amendments to the soil reporting
17 conflicting effects. For example, amending soil with thermophile-fermented compost
18 soil stimulated denitrification (Ishikawa et al. 2012) while addition of olive mill
19 pomace did not affect it (Gomez-Munoz et al. 2011). These findings suggest that
20 substrate composition, soil properties and environmental factors interact and
21 eventually determine N losses from soils amended with organic substrates. Moreover,
22 few studies to date have studied the pattern of denitrifiers in soils amended with
23 organic substrates (Miller et al. 2009) and their interactions with nitrifiers or their
24 potential use of the abundance of denitrifying genes as an indicator for tracing N
25 losses.

1 The objective of this study was to investigate the effect of MSW-compost amendment
2 on ammonia oxidizers and their interrelationships, if any, as well as with denitrifiers.
3 This information could be effectively used to predict and manage N release,
4 availability and losses.

5 **Materials and Methods**

6 Experimental design

7 The study was carried out under controlled conditions in 0.75 L pots filled in with 500
8 g of dry soil. The treatments included pots treated with 0, 50 and 100 tons per hectare
9 (t/ha) MSW-compost derived from Chania Municipality. The composition of MSW-
10 compost was pH: 7.54 ± 0.12 ; electrical conductivity (EC): 0.146 ± 0.08 dS/m; total
11 nitrogen (TN): 3.75%; NH_4^+ -N: 108 ± 14 mg/kg; NO_3^- -N: 1385 ± 217 mg/kg; SOM:
12 $23.73 \pm 2.4\%$. With regard to the trace elements, although their total content was
13 within the typical range reported for MSW-composts, their availability in the
14 amended soil remained very low to cause any effects on microorganisms activity
15 (Giannakis et al., unpublished data). The soil used in this study was sampled from a
16 20-year-old citrus orchard regularly tilled and fertilized and was classified as clay-
17 loam (35% clay, 37% silt, 28% sand) with pH: 7.5, EC: 0.3 dS/m, TN: 0.31%, SOM:
18 1.58%. Before filling in pots, the soil and the compost were passed through a 2mm
19 diameter screen and were thoroughly mixed. Then, the pots were watered to field
20 capacity and transferred in a controlled growth chamber (25 °C) under dark
21 conditions. The pots were arranged in a full randomized design and care was given to
22 maintain soil moisture close to field capacity by weighting some pots every two/three
23 days and replacing water losses.

24 Soil Sampling and Analyses

1 Whole pots were destructively sampled (two pots per treatment) for a period of 125
2 days. The soil samples were treated, prepared and analyzed according to the Methods
3 of Soil Analysis (Methods of Soil Analysis 1982). The particle size analysis of the
4 soil samples was carried out by the Bouyoucos hydrometer method (Bouyoucos
5 1962). The Walkley and Black wet-digestion method was used for the determination
6 of soil organic matter (SOM). Total nitrogen (TN) was measured by an elementary
7 analyser. Ammonium was extracted with 2 M KCl for 30 min and measured
8 colorimetrically in a Perkin-Elmer Lambda 25 spectrophotometer by the Nessler
9 reagent. Nitrates were measured colorimetrically by the Cd reduction method after
10 extraction with distilled water for 2 h.

11 Carbon Mineralization Rate

12 To assess C mineralization rate three pots per treatment were placed in one-litter jars
13 sealed by a gas-tight screw lid. The CO₂ emitted from the pots was trapped in a vial
14 containing 10 ml of 1 M NaOH placed inside the jar. The concentration of CO₂ was
15 assessed by titration. In addition, jars with dry soil were also included to compensate
16 for the effect of atmospheric CO₂. Measurements were taken daily in the first week,
17 every two days in the following two weeks and two times per week thereafter by the
18 end of the study.

19 DNA extraction and qRT-PCR analyses

20 Microbial genomic DNA was extracted from 0.5 g of soil, previously frozen and
21 homogenized with mortar, using the UltraClean Soil DNA Isolation Kit (MO BIO
22 Laboratories, Inc. Carlsbad, CA, USA) according to manufacturer's instructions.
23 DNA quality was checked in agarose gel and was quantified in a Lambda 25
24 spectrophotometer (Perkin Elmer). DNA was diluted in 50 µl and stored at -80 °C.

1 PCR amplification of ammonia oxidizing bacterial (AOB) and ammonia oxidizing
2 archaeal (AOA) *amoA* gene copies was carried out with the primer pairs amoA-
3 1F/amoA-2R (Rotthauwe et al. 1997) and amoAF/amoAR (Francis et al. 2005),
4 respectively with 200 nM primers. The cycling conditions for the amplification of
5 AOB and AOA *amoA* genes was were 3 min at 95 °C, followed by 35 cycles of 30 s at
6 95 °C, 35 s at 57.5 °C (62 °C for AOA) and a data acquisition step at 84 °C and 81 °C,
7 respectively for 15 sec. Amplification of denitrification genes *nirK*, *nirS*, and *nosZ*
8 was performed with the primer pairs nirK876/nirK1040 (Henry et al. 2004),
9 nirSCd3aF/nirSR3cd (Throbäck et al. 2004), and nosZ2F/nosZ2F (Henry et al. 2006),
10 respectively at 500 nM primer concentration. The thermal protocol for the *nirK*, *nirS*,
11 and *nosZ* primers included an initial step of 95°C for 3 min; 35 cycles 95°C for 15 s,
12 60°C for 60 s, followed by a data acquisition data step at 87.5 °C, 84.5 °C and 84.5
13 °C, respectively for 15 sec. Data acquisition temperatures for all the reactions
14 described was assessed by running trial qRT-PCRs including the standards and some
15 “unknown” samples.

16 Quantification of gene copy numbers was performed with the StepOnePlus™ Real-
17 Time PCR System (Applied Biosystems) in 20 µl reactions using the KAPA SYBR
18 Fast Master Mix (2x) qRT-PCR Kit (KAPA Biosystems) and 1 µl of 1/20 diluted soil
19 DNA. SYBR Green dye. All reactions were completed with a melting curve starting
20 at 60 °C with an increase of 0.5 °C up to 95 °C to verify amplicon specificity.
21 Standard curves were obtained using serial dilutions, 10³-10⁸ for ammonia oxidizing
22 organisms and 10²-10⁷ for denitrifiers, of linearized plasmids (pGEM-T, Promega)
23 containing cloned *amoA*, *nirK*, *nirS*, and *nosZ* genes amplified from the soil samples
24 of the present study. Controls without templates resulted in undetectable values in all
25 samples and inhibitory effects on PCR performance were not detected at the dilution

1 used. The amplification efficiencies were 83% for AOB, 90% for AOA, 87% for
2 *nirK*, 78% for *nirS*, and 88% for *nosZ* and the R² values of the standard curves ranged
3 from 0.997 to 0.999.

4 Data analysis

5 Eighteen replications per treatment (compost dose) were included. The t test was used
6 to estimate if compost dose had a significant effect on TN, NH₄⁺-N, NO₃⁻-N,
7 archaeal/bacterial *amoA* gene abundance and denitrifying genes (*nirK*, *nirS*, and *nosZ*)
8 abundance. Pearson's test was employed to test the significance of the correlations
9 observed in the present study. Statistical analysis, including outliers detection, was
10 carried out by SPSS 19.0 software.

11 **Results**

12 Respiration rate

13 Compost amendment stimulated respiration rate, but it did not follow a dose-response
14 pattern (Fig. 1). The highest rates were measured three days after compost
15 incorporation. Thereafter, respiration rates declined to reach a constant rate on Day 20
16 which maintained by Day 77. Then, a second smoother decline in respiration rate was
17 observed by Day 110 (Fig. 1).

18 Soil nitrogen

19 Soil NH₄⁺-N content increased slightly in the first 3 days after MSW-compost
20 incorporation and then maintained constant by day 10. A further increase in soil
21 NH₄⁺-N content occurred in the interval day 10 to 32 for the compost-amended
22 treatments and then it remained constant by day 62, when a decrease was observed by
23 the end of the study (Fig. 2a). In controls, NH₄⁺-N content maintained relatively
24 constant throughout the study period except for the interval, day 92 to 123, when a
25 slow increase took place (Fig. 2a).

1 Soil NO₃⁻-N content increased sharply from day 0 to day 3 in MSW-compost
2 amended treatments and this increase was continued by day 6, although at a lower
3 rate, in the highest compost dose (Fig. 2b). Thereafter, soil NO₃⁻-N content remained
4 constant until day 62 in all treatments in consistency with potential nitrification rates
5 with were zero during this period (data not shown). From this date onwards, soil
6 NO₃⁻-N content increased linearly for the highest MSW-compost treatment (Fig. 2b)
7 while in the 50 t/ha and the non-amended treatments lower rates of NO₃⁻-N
8 accumulation prevailed by day 92 and then nearly ceased by the end of the study (day
9 123).

10 With regard to TN content, no statistically significant changes were observed 62 days
11 after compost incorporation for all the treatments (Fig. 2c). Thereafter, a progressive
12 decline, statistically significant at the non-amended and the amended with 50 t/ha
13 treatments, took place. At the end of the study, the decline of TN compared to its
14 initial values was estimated to be 25%, 15%, and 9% on average for 0, 50 t/ha, and
15 100 t/ha, respectively (Fig. 2c).

16 Ammonia oxidation and denitrification genes abundance

17 Changes in soil NO₃⁻-N content were associated with corresponding changes in
18 archaeal and bacterial *amoA* gene copies (Fig. 3a and 3b). Initially, in the first three
19 samplings carried out on days 3 and 10, and 32, low copy numbers of the archaeal and
20 bacterial *amoA* gene were quantified in all the treatments (Fig. 3a and 3b). On day 62,
21 *amoA* gene copy numbers increased up to one order of magnitude and peaked off by
22 day 92. Overall, no significant differences were found among treatments (compost
23 dose) except in some cases (day 3; day 62). A clear effect of the treatment on archaeal
24 and bacterial *amoA* gene copies was detected in the last sampling (day 122), when the
25 treatments amended with MSW-compost showed higher gene copy numbers

1 compared to the non-amended treatment (Fig. 3a and b). Moreover, the strong
2 correlation between AOA and AOB *amoA* copies (R^2 : 0.94; $P < 0.001$) and the high
3 slope of the curve (13) reveals that AOA responded more rapidly than AOB to
4 ammonia availability (Fig. 3c).

5 The variation of denitrifying gene copy numbers was assessed on days 3, 32, 62, 92
6 and 123. Overall, they followed the general trend observed in the case of ammonia
7 oxidizers with low copy numbers by day 32 followed by an increase up to one order
8 of magnitude on day 62. Treatments (compost dose) had only a slight influence on
9 denitrifiers copy numbers ((Fig. 4a; 4b; and 4c). More detailed, higher numbers of
10 *nirK* copies were assessed in the highest compost treatment compared to the non-
11 amended soil on day 32 and 92 (Fig. 4a). A similar effect was also observed for *nirS*
12 and *nosZ* denitrifiers on day 92. Moreover, *nirK* denitrifiers responded more to MSW-
13 compost than to *nirS* nitrifiers (Fig. 4a and 4b). *nosZ* denitrifiers followed strictly the
14 pattern of *nirK* denitrifiers. Strong correlations were established between ammonia
15 oxidizers, both archaeal and bacterial, and denitrifiers (Fig. 5a-5d).

16 **Discussion**

17 Compost incorporation resulted in diverse patterns of nitrification and denitrification
18 throughout the period of the study. Initially (day 0 to 6), soil NO_3^- -N content
19 increased in MSW-compost treated soils due to the NH_4^+ -N contained in the compost
20 and the mineralization of easily degradable substrates which released NH_4^+ -N
21 subsequently oxidized by ammonia oxidizers. This effect was consistent with the
22 greater respiration rates prevailed during the first days of the study (Fig. 1). During
23 this period the greater rates of NH_4^+ -N release probably enabled nitrifiers to compete
24 with heterotrophs for the available NH_4^+ -N. Then, and until day 62, soil NO_3^- -N and
25 NH_4^+ -N content maintained relatively constant providing evidence that N

1 immobilization took place. Immobilization of N has been common in soils amended
2 with MSW-compost (Busby et al. 2007; García-Gil et al. 2000). However, a similar
3 response observed in the non-amended soil suggesting that the soil also contributed to
4 immobilization. Indeed, clayey soils stimulate increases in the abundance of
5 microorganisms favoring N immobilization (García-Gil et al. 2000; Madrid et al.
6 2011). Likewise, accumulation of NO_3^- -N occurred only 12 weeks after MSW-
7 compost incorporation in a clayey soil (Madrid et al. 2011).

8 From day 10 to 32 soil NH_4^+ -N slightly increased in compost amended soils but at the
9 same period archaeal and bacterial *amoA* gene copies remained constant and at levels
10 similar to these of the non-amended soil and net potential nitrification rates remained
11 close to zero. This pattern may suggest antagonism with heterotrophic bacteria or
12 fungi for the available O_2 supply which out-competed the growth and the activity of
13 ammonia oxidizers or raises issues related with NH_3 availability to nitrifiers. The
14 increase in the population of ammonia oxidizers that took place on day 62, just before
15 the increase of soil NO_3^- -N content, may indicate that N immobilization had ceased
16 and this hypothesis is further supported by the recovery of potential nitrification rate.
17 These findings suggested that changes in the abundance of *amoA* gene copies could
18 be used as an indicator for tracking changes in soil nitrification potential.

19 The treatments imposed in this study (0, 50, and 100 t/ha MSW-compost) did not
20 change significantly the *amoA* gene copies of AOA and AOB, except in the last
21 sampling, (Fig. 3a; b) but on the other hand differentiated net nitrification rates (0.71
22 to 1.30 mg N/kg soil d) especially in the interval 62 to 123 day. This may due to the
23 fact that abundance of functional genes is not a precise measure and provides only
24 limited information for the activity of AOA and AOB, since the process is regulated
25 at the level of transcripts and the enzyme activity and as a consequence, consistent

1 patterns are rarely obtained (Prosser and Nicol 2012). Likewise, conflicting or no
2 relationships have been often reported between *amoA* gene abundance and potential
3 nitrification rate (Di et al. 2009; Jia and Conrad 2009; Petersen et al. 2012).

4 Under conditions of low NH₃ availability an advantage of AOA over AOB has been
5 reported (Levicnik-Hofferle et al. 2012). Application of biosolids at the rate of 27 t/ha
6 stimulated the growth of AOA only, but increasing application dose to 54 t/ha resulted
7 in the growth of both AOA and AOB (Kelly et al. 2011), an effect similar to that
8 observed in this study. The greater increase of AOA abundance that preceded the
9 recovery of nitrification, as indicated by the high slope (13) of the correlation curve
10 between AOA and AOB *amoA* gene copies (Fig. 3c), provided evidence for the
11 involvement of AOA in the oxidation of ammonia and their advantage over AOB
12 under conditions of low ammonia release. Strong evidence in favor of the argument
13 that NH₃ concentration determines differential growth of AOA and AOB was recently
14 published (Xu et al. 2012).

15 Amendment of soils with organic substrates is often accompanied by increased
16 denitrification rates and losses of N (Ishikawa et al. 2012) but it is not always the
17 case, since interactions between C availability and composition and NO₃⁻-N
18 availability regulate N emissions (Miller et al. 2009; Verhamme et al. 2011). In this
19 study copy numbers of the genes involved in denitrification maintained at relatively
20 low levels until day 32 (Fig. 4) suggesting the prevalence of low rates of
21 denitrification and this hypothesis is consistent with the similar soil TN content
22 measured during this period (Fig 2c). This finding was somewhat unexpected given
23 the relatively high soil NO₃⁻-N content, especially in compost-amended soils, the
24 relatively high SOM, and the texture of the soil that favored locally the prevalence of
25 anoxic conditions. Application of olive mill pomace to the soil also resulted in low

1 N₂O emissions suggesting the prevalence of low denitrification rates which were
2 attributed to the recalcitrant nature of compost (Gomez-Munoz et al. 2011). A gradual
3 decrease in the soil TN content started on day 62 and accompanied by a strong
4 increase in denitrifying genes abundance implying N losses through denitrification.
5 Surprisingly, the proportion of N lost at the end of the study was inversely related to
6 the compost dose and soil NO₃⁻-N content. This finding contrasts with gene copy
7 numbers of denitrifiers which in overall did not differ significantly between
8 treatments or were slightly higher in MSW-compost amended treatments (Fig. 4).
9 Probably the composition of organic matter (Wu et al. 2012) in the compost amended
10 soil and/or competition with obligate aerobic heterotrophs suppressed denitrification
11 activity. Likewise, although the application of liquid dairy manure and liquid swine
12 manure induced denitrifying gene copies this increase was not associated with
13 denitrifying activity (Miller et al. 2009). Weak correlations between abundance of
14 denitrifying genes and activity is a common observation and has been attributed to
15 their variable response to environmental stimuli, and the fact that the currently used
16 primers may do not provide an accurate picture of the abundance of denitrifying genes
17 (Dandie et al. 2008; Miller et al. 2008). Moreover, fungi have been found to
18 contribute to soil denitrifying activity but their contribution has not been quantified so
19 far (Shoun et al. 2012). Another important issue is the similar numbers of *nosZ* genes
20 detected in all the treatments to those of *nirK* which may imply low potential for N₂O
21 emissions. Such a hypothesis is, however, very simplistic, since many studies have
22 failed to establish a link between *nosZ* genes abundance and N₂O emissions (Braker
23 and Conrad 2011; Dandie et al. 2011). Moreover, a recent study challenged the
24 abundance of typical NOS proteins to provide information on the potential of soil to
25 reduce N₂O to O₂ due to the presence of divergent *nos* genes in diverse microbial taxa

1 that are evolutionarily distinct from the typical *nos* genes of denitrifiers (Sanford et al.
2 2012). Apparently, direct measurements are required to gain insights on the effect of
3 MSW-compost on N₂O emissions. Moreover, the strong relationships (P<0.01)
4 between ammonia oxidizers and denitrifiers (Fig. 5) imply a tight communication and
5 interdependence among them which cannot be explained only by substrates
6 availability. In situ probing studies are required to provide insights on these microbe-
7 microbe interactions, the distribution of microorganisms with the availability of
8 resources, and the effect of environmental conditions. This need is also stressed by
9 Prosser and Nicol (2012) who suggested that analysis of soil heterogeneity and micro-
10 environments is required to elucidate the factors regulating the community
11 composition and activity of ammonia oxidisers.

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1 **Figure captions**

2 Figure 1: Respiration rate of a clay-loam soil treated with 0, 50, and 100 t/ha MSW-
3 compost.

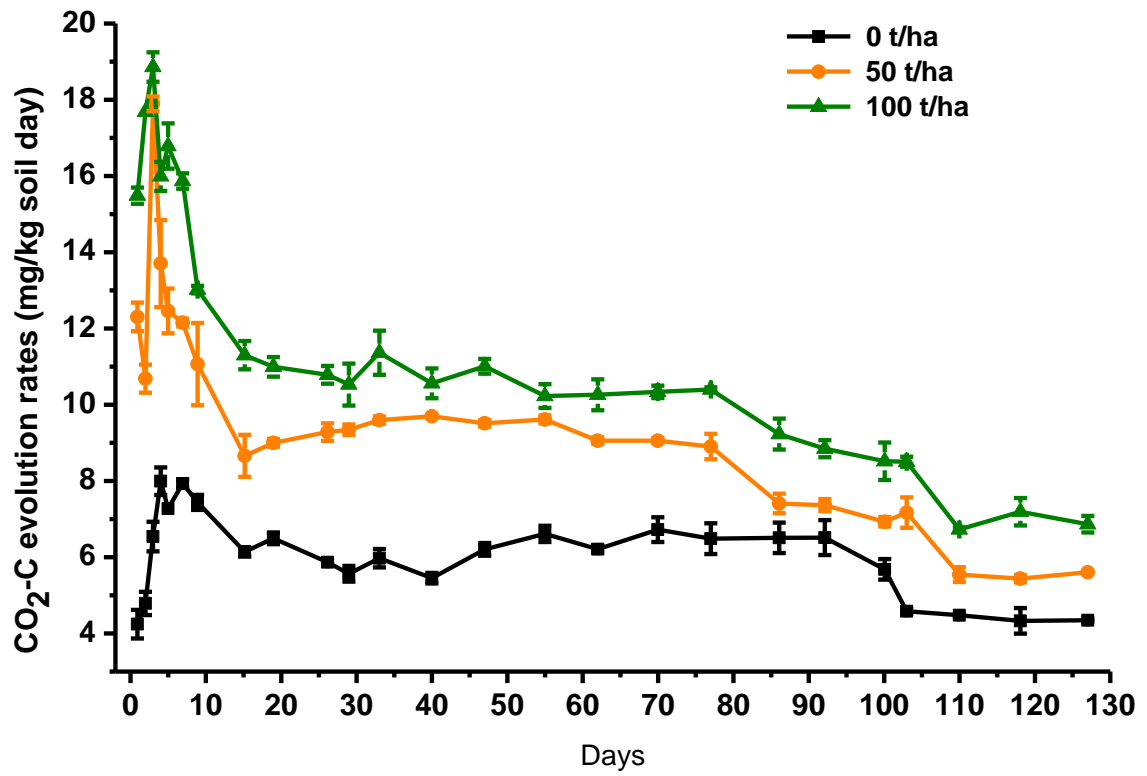
4 Figure 2: Variation of soil NH_4^+ -N content (a); soil NO_3^- -N content (b); and soil total-
5 N content throughout the study period in a clay-loam soil treated with 0, 50, and 100
6 t/ha MSW-compost.

7 Figure 3: Changes in archaeal *amoA* gene copies (a) and bacterial *amoA* gene copies
8 throughout the study period in a clay-loam soil treated with 0, 50, and 100 t/ha MSW-
9 compost (b). (c) Correlation of AOA *amoA* vs AOB *amoA* (R^2 : 0.946; $P < 0.01$).

10 Figure 4: Denitrifying genes copy numbers variation of soils amended with 0, 50, and
11 100 t/ha MSW-compost throughout the course of the study; *nirK* gene copy numbers
12 (a), *nirS* gene copy numbers (b), and *nosZ* gene copy numbers (c).

13 Figure 5. Correlations of (a) archaeal *amoA* versus *nirK* (R^2 : 0.946; $P < 0.01$), (b)
14 bacterial *amoA* versus *nirK* (R^2 : 0.820; $P < 0.01$), (c) archaeal *amoA* versus *nirS* (R^2 :
15 0.915; $P < 0.01$), and (d) bacterial *amoA* vs *nirS* (R^2 : 0.823; $P < 0.01$) in soils amended
16 with 0, 50, and 100 t/ha MSW-compost.

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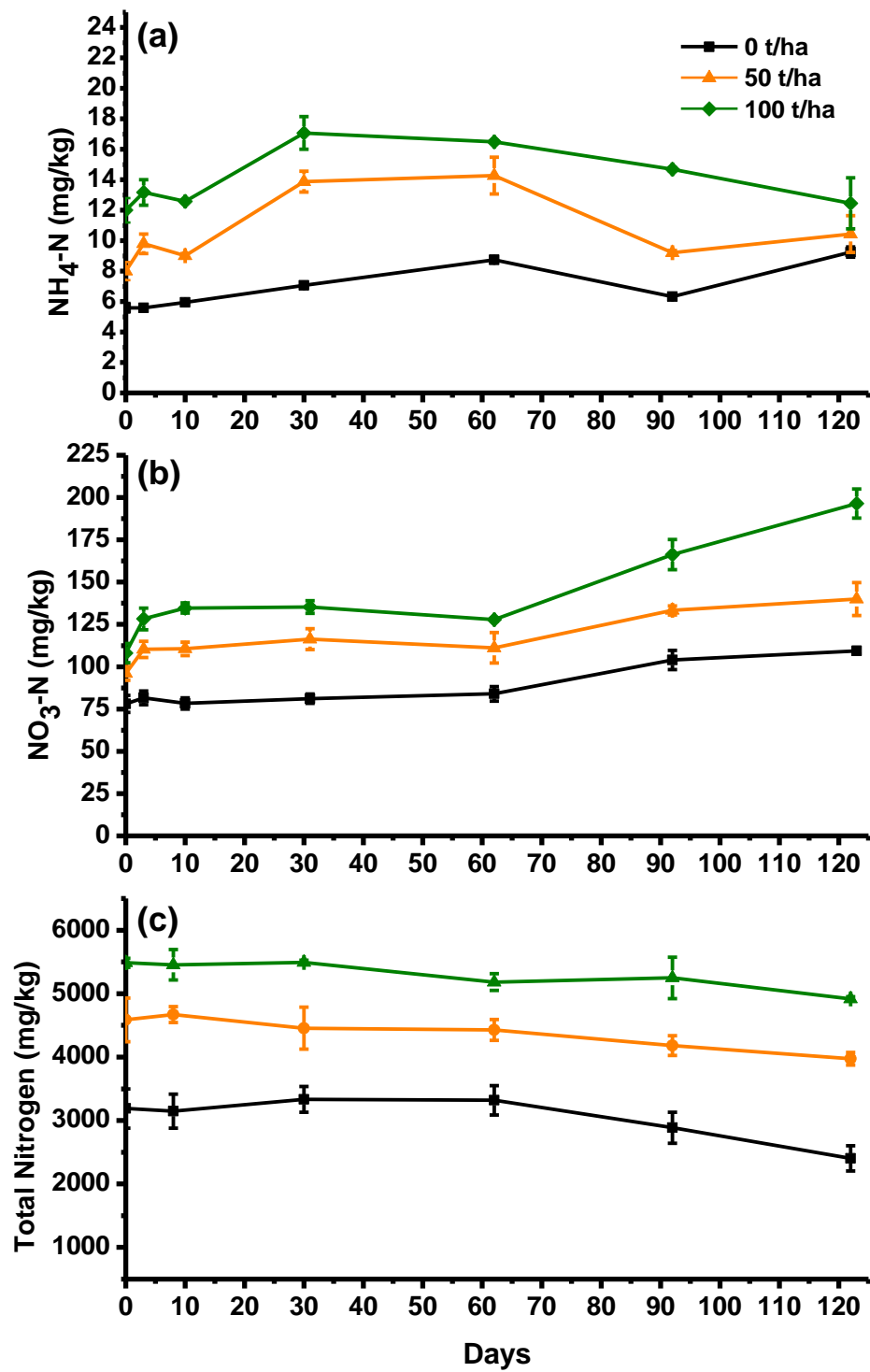


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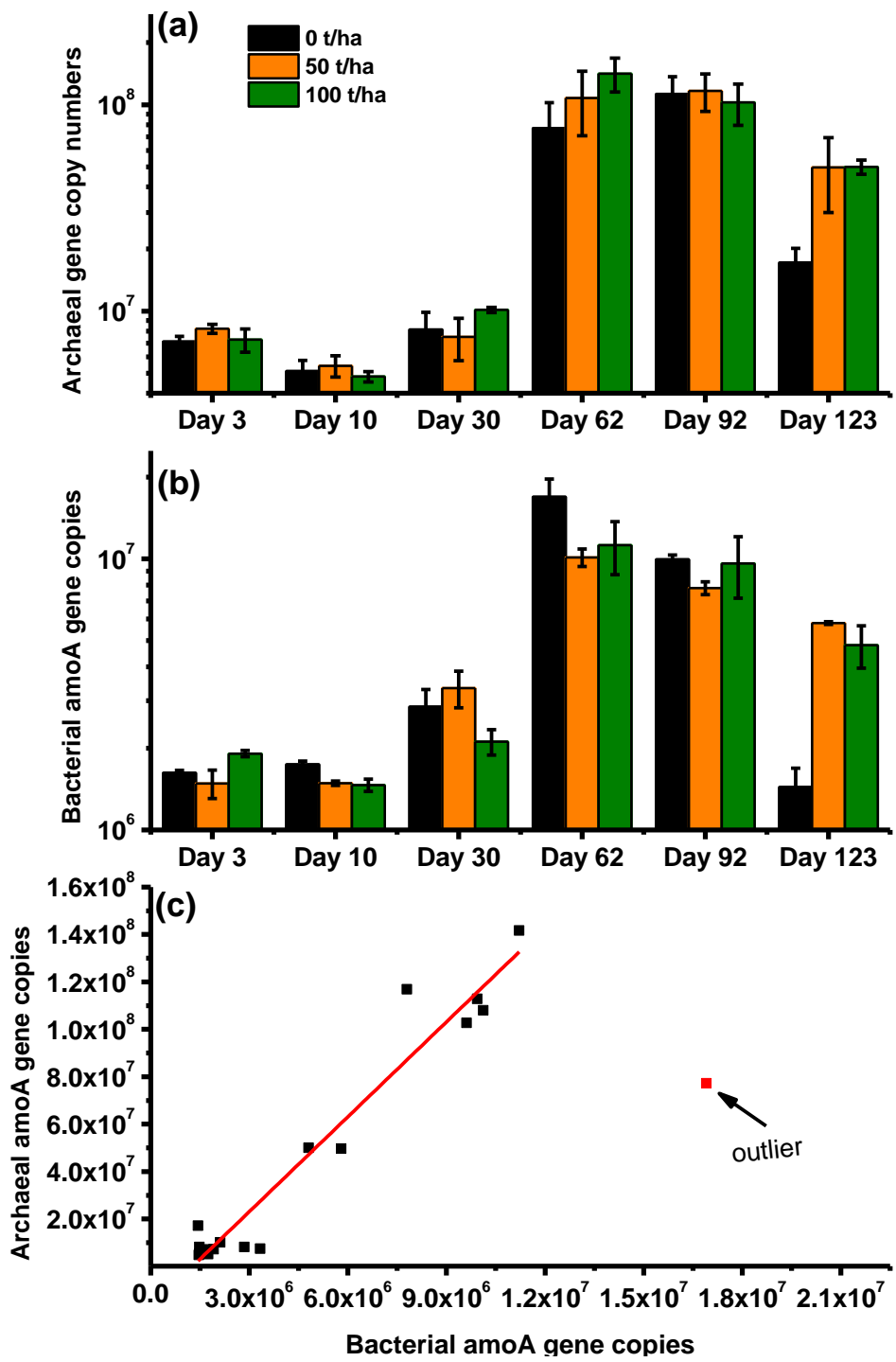


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3 Figure 2: Variation of soil $\text{NH}_4^+\text{-N}$ content (a); soil $\text{NO}_3^-\text{-N}$ content (b); and soil total-
4 N content throughout the study period in a clay-loam soil treated with 0, 50, and 100
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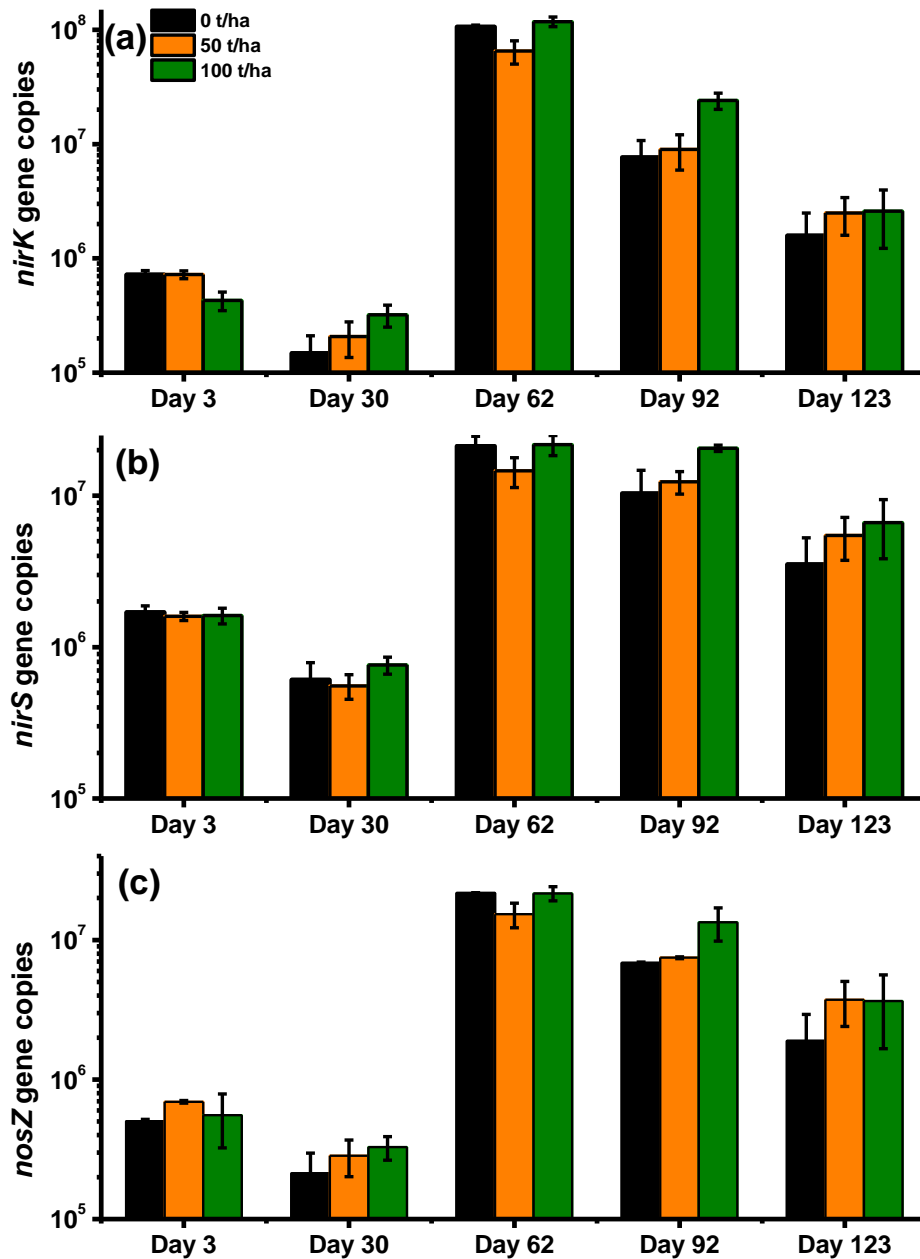
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3 Figure 3: Changes in archaeal *amoA* gene copies (a) and bacterial *amoA* gene copies
4 throughout the study period in a clay-loam soil treated with 0, 50, and 100 t/ha MSW-
5 compost (b). (c) Correlation of AOA *amoA* gene copies vs AOB *amoA* gene copies
6 (R²: 0.946; P<0.01).

7



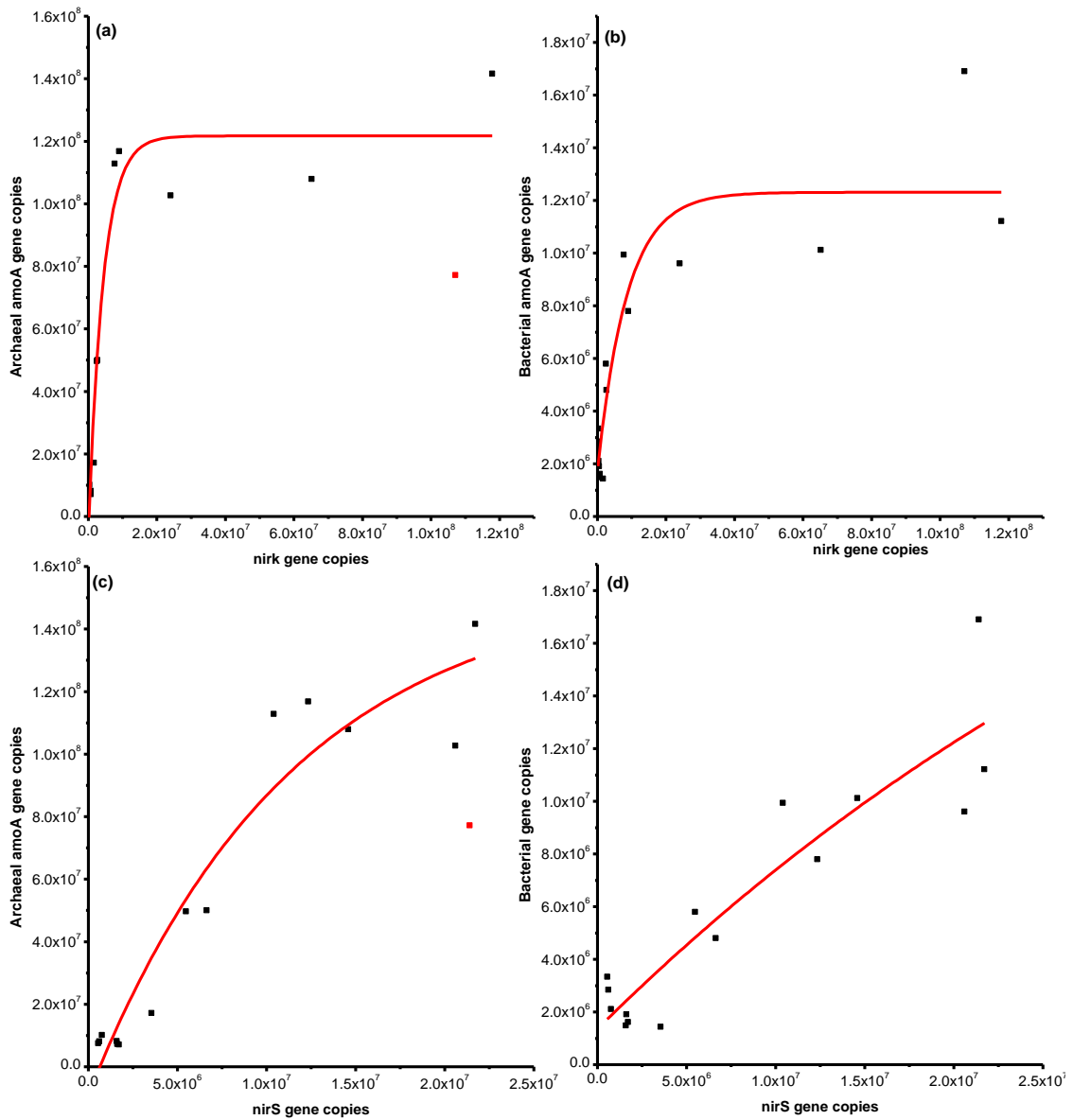
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4 bacterial amoA versus nirK (R^2 : 0.820; $P < 0.01$), (c) archaeal amoA versus nirS (R^2 :
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