6 Response to nitrogen-cycling functional genes to the application of biochar, compost and their mixture[4]

The submitted article is presented below. Although it is also presented in deliverable 5-3 on pot experiments, it allows to present the properties of the organic amendments tested and in particular the microbial communities present which play a role in the nitrification and denitrification processes of the nitrogen cycle. An extended abstract and the graphical abstract are presented just below. The submitted article is presented in extenso after the extended abstract.

Microbial communities play an important role in soil nitrogen (N) cycling in many agroecosystems. However, how organic amendments influence their abundance and interaction with soil properties is little studied in soils of arid regions. The objective of this study was to evaluate changes in functional N-cycle genes involved in nitrification and denitrification in a sandy soil.

Compost amendments showed the highest bacterial abundance followed by biochar-compost amendments, which also significantly increased 16S gene abundance. Soils amended with biochar, urea, or biochar-urea showed no significant differences compared to non-amended soils. Fungal abundance was highest with compost. The fungi-to-bacteria ratio was low (0.005–0.01), likely due to the sandy texture of soil and faster bacterial recovery. Compost, with a low C/N ratio, favored bacteria over fungi. The low organic carbon content of the soil limited fungal development. Organic matter quality has been shown to influence the fungi/bacteria ratio, with low-quality substrates (high C/N ratio) promoting fungi and high-quality substrates (low C/N ratio) favoring bacteria (Six et al., 2006). In this study, compost with a low C/N ratio, as shown in Le Guyader et al., (2024), stimulated bacterial growth more than fungal growth.

Ammonia-oxidizing archaea (AOA) gene copy numbers significantly increased with all amendments except urea, with compost showing the highest abundance. Ammonia-oxidizing bacteria (AOB) abundance was only significantly higher with compost, likely due to its higher initial ammonium levels. The AOA-to-AOB ratio was highest in non-amended soil, indicating a greater role of AOA in nitrification, while most amendments decreased this ratio except for biochar. These shifts reflect the influence of soil properties, such as nitrogen availability, on AOA and AOB dynamics. High N amendments like compost and urea favored AOB, aligning with studies showing their preference for N-rich conditions.

Denitrifiers were evaluated through nirK, nirS, and nosZ genes. The highest abundances of these genes were observed with compost or biochar-compost amendments, indicating an enhanced potential for denitrification and efficient nitrate conversion to N_2. Compost alone significantly increased denitrifying communities, while mixing compost with biochar reduced

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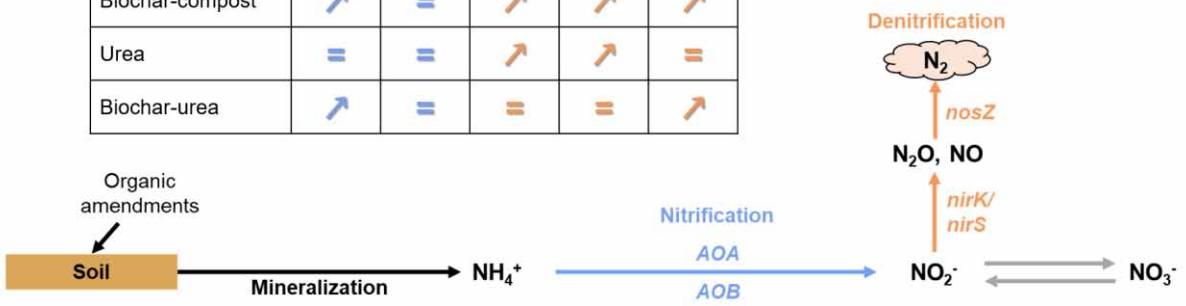
their abundance, aligning with biochar's role in reducing N_2O emissions. Biochar, particularly at 550°C, has been shown to reduce N_2O emissions by altering pH and retaining nitrogen, although its effect on denitrification can be transient. Biochar alone had minimal impact on denitrifying gene abundances, except for nirS. These findings highlight biochar's potential to mitigate N losses while influencing microbial dynamics.

Variation in N-cycle gene abundances in comparison to non-amended soil

Amendments	*AOA*	*AOB*	*nirK*	*nirS*	*nosZ*
Biochar	↗	=	=	↗	=
Compost	↗	↗	↗	↗	↗
Biochar-compost	↗	=	↗	↗	↗
Urea	=	=	↗	↗	=
Biochar-urea	↗	=	=	=	↗



Article Title: Response of nitrogen-cycling functional genes to the application of biochar, compost, and their mixture in sandy soils under arid conditions

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Abstract

Microbial communities play an important role in soil nitrogen (N) cycling in many agroecosystems. However, how organic amendments influence their abundance and interaction with soil properties is little studied in soils of arid regions. The objective of this study was to evaluate changes in functional N-cycle genes involved in nitrification and denitrification in a sandy soil. The soil was amended with organic products obtained from date palm residues and analysed after 4 months of barley cultivation under arid climate. The amendments included urea (U), biochar (BC), compost (C), biochar-urea (BCU), biochar-compost (BCC), and non-amended soil as control. Key genes involved in nitrification, including archaeal (amoA AOA) and bacterial (amoA AOB) amoA genes, and denitrification (nirK, nirS, nosZ) processes were quantified using qPCR along with bacterial and fungal abundances. Soil properties and parameters related to soil N dynamics were also determined. Results indicated that bacterial abundance was higher than fungal abundance in amended and non-amended soils. They also showed a large variation in the abundance of AOA and AOB communities, with the dominance of AOA over AOB in non-amended soil, BC and urea amended soils. Compost amendment significantly increased the abundance of nirK, nirS, and nosZ denitrifier communities. This increase was positively correlated with total NO3- in leachates, soil NH4+ concentration, and total mineral N in the soil. However, when compost was combined with biochar, there was a reduction in the abundance of the denitrifying communities suggesting that mixing compost with biochar may reduce N loss caused by denitrifying communities.

Keywords: Nitrogen, N-cycling, nitrification, denitrification, microbial communities, biochar, compost

6.1 Introduction

The recycling of crop residues to produce organic amendments (OA), such as biochar and compost, can help enhance soil fertility, particularly in arid regions where nitrogen (N) availability limits crop production (Robertson and Vitousek, 2009). This practice also contributes to improving environmental quality, especially in arid regions where natural resources are limited (El Janati et al., 2022). In arid regions, where soils have low fertility and

limited available water capacity, soil microorganisms play an important role in N transformations (Robertson and Vitousek, 2009; Sinsabaugh et al., 2015).

Soil management practices are often implemented to enhance N availability for crop uptake while simultaneously reducing N leaching and greenhouse gas emissions (Robertson and Vitousek, 2009). OMs are commonly applied to increase soil organic content and decrease the cost related to the use of mineral fertilizers (Robertson and Vitousek, 2009). Biochar is a carbon-rich product produced by the pyrolysis of organic matter in the absence of O2 (Khan et al., 2023). In comparison to compost, biochar has a higher carbon content and its porous structure can promote water retention (Jien et al., 2015; Khan et al., 2023). Biochar and compost are known to influence the availability of N and nutrients in soil, but their effect will depend on their properties (e.g. type of residues, N content), on soil characteristics (e.g. pH, moisture, particle size) and on climatic conditions (Pereg and McMillan, 2020; Kavvadias et al., 2024). Unlike mineral fertilizers, which provide available N rapidly for uptake by plants, organic amendment must be mineralized by microorganisms before their N becomes available for plant uptake (Robertson and Vitousek, 2009; Song and Niu, 2022). However, despite the importance of OA for soil in arid regions, our understanding of the processes governing N transformations in arid soils remains limited (Arfaoui et al., 2019; Jansson and Hofmockel, 2020).

Nitrification and denitrification are two major N-cycling processes. Nitrification involves the conversion of ammonium ions (NH4+) to nitrite (NO2−) and then to nitrate (NO3−) (Schimel and Bennett, 2004). It has been shown that the oxidation of ammonium ions is the rate-limiting step in the overall nitrification process (Robertson and Vitousek, 2009). Nitrification contributes to N plant nutrition and without it little nitrate would be available for root plant uptake (Robertson and Vitousek, 2009; Zheng et al., 2022). On the other hand, denitrification involves the reduction of nitrate to gaseous forms such as nitric oxide (NO), nitrous oxide (N2O), and nitrogen gas (N2) (Schimel and Bennett, 2004). Understanding and predicting the response of soil microbes involved in nitrification and denitrification to OA, such as biochar and compost, could help improve N-use efficiency in arid agroecosystems (Jansson and Hofmockel, 2020).

In most cases, the application of OA stimulates microbial activity and many studies have shown an increase in both nitrification and denitrification rates under temperate climate (Maeda et al., 2011; Blaud et al., 2018; Liao et al., 2020). Nevertheless, the influence of OA on the abundance of N-cycling microbial communities in soils of arid regions remains understudied (Sinsabaugh et al., 2015). The use of molecular markers targeting functional genes can provide indication of soil N-cycling potential, as demonstrated in various ecosystems (Fierer et al., 2005; Pereira et al., 2015; Pereg and McMillan, 2020). Therefore, the objectives of this study were to evaluate changes in the abundance of microbial communities involved in nitrification and denitrification in a sandy soil with similar properties to those found in arid regions of North Africa. The soil was amended with biochar, compost, urea, and their combinations, and analyzed after four months of barley cultivation under arid climate. The amoA gene was used as a molecular marker for nitrifier communities (ammonia oxidizers), while the nirK, nirS, and nosZ genes were employed to assess the denitrification process (Blaud et al., 2018; Liao et al., 2020). Our initial hypothesis is that combining biochar with compost would promote microbial growth in comparison to biochar or compost alone, which will result in an increase in total bacterial and fungal abundance. Additionally, we hypothesize that biochar alone will have a more moderate

effect on microbial growth due to its lower N content compared to compost. Given the nutrient richness of compost, including N, as showed in Le Guyader et al. (2025), we hypothesized that the abundance of nitrifiers and denitrifiers would increase all compost containing soil in comparison to the non-amended soil. However, when biochar is mixed with compost, their abundance would decline due to the enhanced retention of N compounds on the biochar surface (Pereira et al., 2015).

6.2 Material and methods

6.2.1 Soil sampling and characterization

Soil samples were collected from soil columns amended with various organic amendments produced from date palm residues and cultivated with barley for 125 days at CEREEP - Ecotron Ile-De-France (ENS CNRS UAR 3194). The soil was sampled in the semi-arid region of Murcia (Spain) in March 2022 and was classified as a Fluvic Gypsic Sodic Solonchak (IUSS Working Group WRB, 2022). To obtain a soil with texture representative of the dominant soils in arid regions, characterized by sandy texture and low levels of organic matter, sand content was increased by supplementing the original soil with quartz sand as described in Le Guyader et al. (2025). The final soil composition was a mixture of 1/4 original soil and 3/4 sand.

Biochar from date palm residues (rachis) was obtained by slow pyrolysis under constant nitrogen flow at a temperature of 450 °C ±5 °C at LERMAB (Laboratory for Studies and Research on Wood Materials) in Épinal (France). The biochar was finely ground and sieved to a size of less than 1 mm. The compost was produced from dry rachis and crushed leaves of date palm (Phoenix dactylifera L.), mixed with 30% sheep manure (v/v). After 3 months of composting, the product was sieved to <4 mm and stored at 4°C until use.

Barley was cultivated in an ecotron for 4 months under controlled conditions simulating the arid climate of the El Atilet oasis in Kebili, Tunisia. The conditions were based on the average data recorded over five years (2017-2021). The weather data were obtained from the National Aeronautics and Space Administration (NASA) Langley Research Center (LaRC) Prediction of Worldwide Energy Resource (POWER) Project. Air temperature ranged from 3.0 to 30.0°C, and relative humidity ranged from 17.0 to 95.6% (Le Guyader et al., 2025).

The soil received organic amendments including urea (U), biochar (BC), compost (C), biochar-urea (BCU), and biochar-compost (BCC), with non-amended soil serving as the control. Each modality was performed in four replicates. Biochar was applied in BC, BCU, and BCC at a dose of 10.4 t/ha, compost in C and BCC at 37 t/ha, and urea at 65 kg N/ha in U. These application rates correspond to doses that are used under field conditions in oasis.

Watering was conducted manually by flooding with a 5 cm layer (1.4 liters of tap water) as it is the local irrigation practice in the oases. Initially, watering occurred every 10 days after plants reached 15 cm height, then increased to weekly after the flowering stage. To estimate nitrogen leaching in the soil, leachates were collected 2 to 5 days after each watering event using 1.5-liter bags. Over the growth period, leachates were collected at 11 time points. For each point, the concentrations of $NO3^-$ were analyzed using a Dionex ICS2000 ion chromatograph system, and $NH4^+$ concentrations were measured with a Seal-A3 HR autoanalyzer. These concentrations were used to calculate the total leaching of each element.

A total of 24 soil samples with organic amendment (6 treatments x 4 replicates) were collected after barley harvest. Each sample was homogenised and sieved through a 2 mm mesh to obtain

representative samples. Subsequently, the soil samples were kept on ice for preservation during transportation to the laboratory, where they were then stored at -20°C awaiting further analysis. Detailed information regarding soil properties and chemical analysis methods can be found in Le Guyader et al., (2025). Soil properties such as pH, electrical conductivity, dissolved organic carbon, and extractable mineral nitrogen concentrations (NH4+, NO3-) were measured in four replicates at post-harvest stage.

6.2.2 DNA extraction

Genomic DNA extraction from both soil and organic products was conducted using the DNeasy PowerLyzer PowerSoil Kit (QIAGEN), following the manufacturer's instructions. A modification to the protocol included an extension of vortexing time from 10 minutes to 20 minutes to improve DNA recovery. Also, the elution volume in the final step was reduced to 50 µL. Quantification of DNA was performed using a NanoDrop Spectrophotometer (NanoDrop OneC, Thermo Scientific™). Assessment of DNA quality was based on the ratios of absorbance at 260 nm/280 nm and 260 nm/230 nm, using the same spectrophotometer. All extracted DNA samples were stored at -20 °C. Genomic DNA was diluted with ultra-pure water to a concentration of 9.6 ng µL−1 for further use.

6.2.3 Real-Time qPCR conditions

Microbial abundance was investigated using quantitative PCR (q-PCR), targeting specific genes or genetic regions important in the N cycle (Fierer et al., 2005). Nitrification processes were explored by targeting both the ammonia-oxidizing archaea (AOA) and bacteria (AOB) through the ammonia monooxygenase subunit A (amoA) gene (Liao et al., 2020; Duff et al., 2022). Denitrifiers were evaluated through the nirK gene, encoding the copper-containing nitrite reductase, the nirS gene encoding the cytochrome cd1-containing nitrite reductase, and the nosZ gene encoding the nitrous oxide reductase (Blaud et al., 2018). Bacterial abundance was quantified using the 16S rRNA genes and the fungal abundance was determined by targeting the ITS gene markers (Blaud et al., 2018). Primer sequences and details of PCR reactions are summarized in Supplementary Table 1.

Quantitative RT-PCR assays were conducted on a CFX Opus 384 instrument (Bio-Rad), using qPCRBIO SyGreen Blue Mix Lo-Rox (Eurobio Scientific) in white 384-well plates (Brand).

All qPCR reactions were performed in a final volume of 5 µl, containing 1 µL of ½ diluted DNA template, 2.5 µL of SyGreen Blue Mix, 0.3 µL of primer (0.6µM for all genes except B16S, which was at 0.1 µM), and PCR-grade water to adjust to 5 µL. The cycling conditions for all reactions consisted of an initial polymerase activation step at 95 °C for 3 min, followed by cycles including denaturation at 95 °C for 5 s, annealing at primer-specific temperatures (refer to Table 1) for 15 s, and elongation at 72 °C for 60 s. Data were acquired at the end of this elongation step. A final step was added to obtain a specific denaturation curve up to 95°C with increments of 0.5°C s−1. Purity of the amplified products was checked by observation of a single melting peak. Subsequently, the amplicons were subjected to analysis via gel electrophoresis (in 2% agarose), stained with ethidium bromide, and visualized under UV light to check for amplicon fragment length. Additional details regarding standard curves and determination of determine the gene copy numbers are described in the Supplementary material.

6.2.4 Statistic and data analysis

Raw results were processed using the software Bio-Rad CFX 1.1 before statistical analysis. The number of gene copies per ng of DNA derived from the real-time qPCR measurements were converted to a number of gene copies per g of dry soil to allow the comparison between soil samples. The Fungi/Bacteria ratio (F/B ratio) was calculated by dividing the number of ITS gene copies by the number of 16S gene copies. Data analysis and graphs were performed using R (R Core Team, 2023). One-way ANOVA was applied to assess differences in microbial gene abundance across amendments, followed by an LSD-test at a significance level of $p < 0.05$. Correlations to test the relationship between soil properties, parameters and nitrogen cycle genes were performed using the corrplot package.

6.3 Results and Discussion

Previous studies have shown that microbial communities can undergo rapid changes within days following the application of organic amendments (Cayuela et al., 2013; Fu et al., 2020; Yang et al., 2022). Contrarily, our study focuses on longer-term effects, investigating changes in N-cycling functional gene abundances four months after the application of amendments, over the whole barley growth cycle. This is an important consideration as N transformations during the subsequent growing period would depend on the abundance and activity of N-cycling microbial communities.

6.3.1 Impact of amendment on bacterial and fungal abundances

Among all amendments, the number of 16S gene copies and therefore bacterial abundance, was highest in the compost amendments (5.18 × 109 copies g-1 dry soil, Figure 20a). BCC amendment also significantly increased B16S gene abundance ($p < 0.05$) compared to the non-amended soil. Non-significant differences were observed between non-amended soil and the soil amended with biochar, urea or biochar-urea. The fungal ITS abundance was also significantly higher ($p < 0.05$) in the compost treatment (5.10 × 107 copies g-1 dry soil) compared to the non-amended soil while biochar-urea amendment showed the lowest fungal abundance among the other amendment (1.32 × 107 copies g-1 dry soil). These values are within the range of those found in previous studies in sandy soils treated with N fertilizer (Fu et al., 2020) or under cropland cultivation (Blaud et al., 2018). The application of organic amendments did not cause a large variation in the fungi to bacteria ratio, as there was only one significant difference between the non-amended soil and the soil amended with urea (Figure 20c). This result indicates that the bacterial communities dominated the studied soil, as the ratio of fungi to bacteria ranged from 0.005 to 0.01 across all amendments. The very low fungi-to-bacteria ratio observed may be due to the initial mixing of the soil with sand to reproduce Saharan soil texture. This reduced both bacterial and fungal populations proportionally but was likely followed by a recovery period during which bacterial populations increased more significantly than fungal populations due to their faster growth rates (Jansson and Hofmockel, 2020), leading to the observed lower fungi-to-bacteria ratio. Organic matter quality has been shown to influence the fungi/bacteria ratio, with low-quality substrates (high C/N ratio) promoting fungi and high-quality substrates (low C/N ratio) favoring bacteria (Six et al., 2006). In this study, compost with a low C/N ratio, as shown in Le Guyader et al., (2025), stimulated bacterial growth more than fungal growth. In addition, the low organic carbon content of the soil was not a factor favoring fungal development. This is consistent with our study, as the studied soil had very low organic carbon content, ranging from 0.53% in non-amended soil to 0.91% in compost-amended soil (Le Guyader et al., 2025).

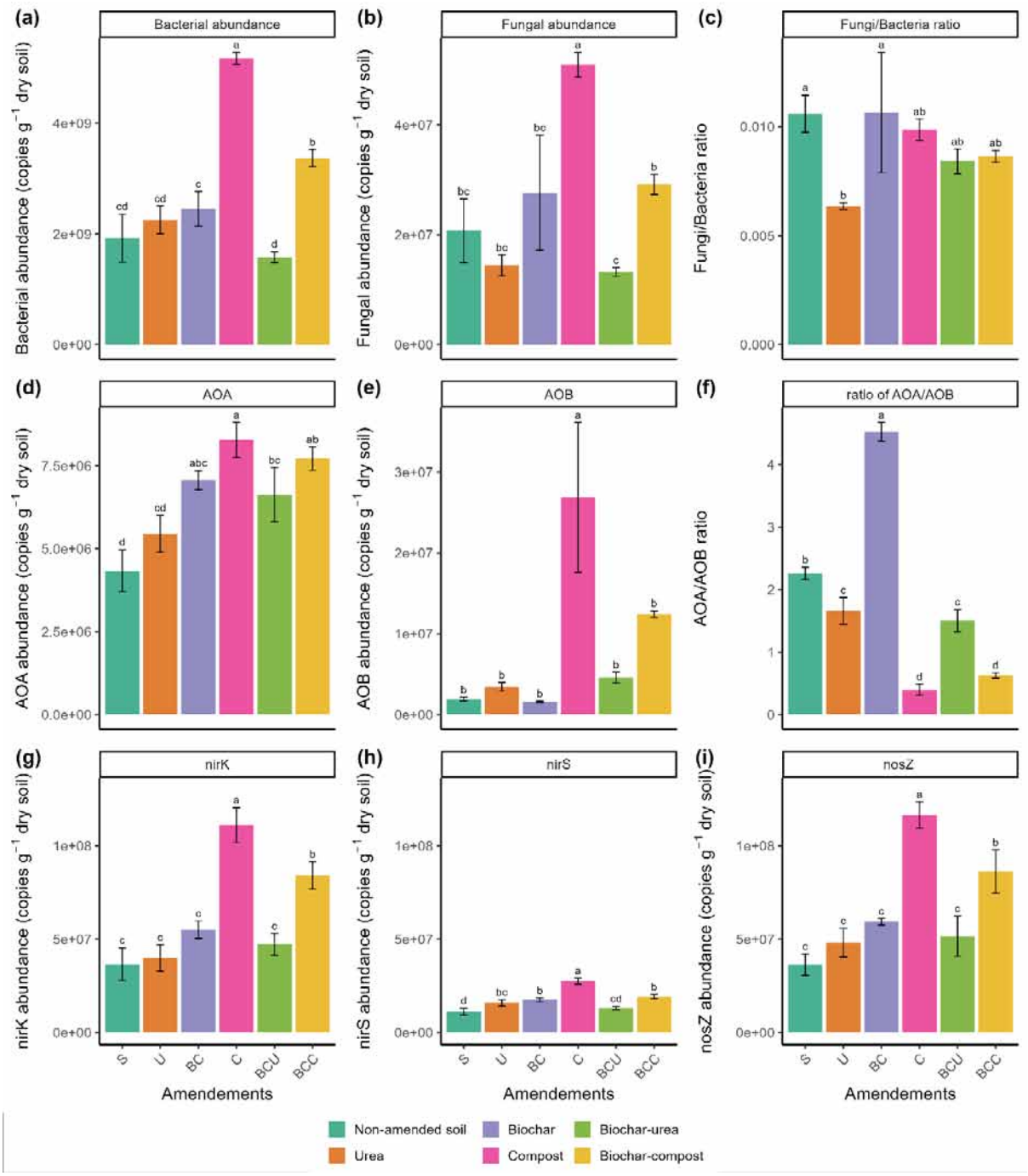

Figure 20 : Variation in gene abundance of total bacteria, fungi, and the fungi/bacteria ratio (a-c), as well as the abundance of AOA, AOB gene and the AOA/AOB ratio (d-f), and the abundance of nirK, nirS, and nosZ genes (g-i) among soils with different organic amendments after four months of barley cultivation. The abundances of microbial communities are expressed as gene copies per gram of dry soil. Different letters indicate significant differences among amendments according to the LSD-test at $p < 0.05$. Values represent means ± standard error of 3-4 replicates. Note that different scales were used on the y-axis.

6.3.2 Genes abundances related to nitrogen nitrification processes

A significant increase in AOA gene copy numbers per gram of soil was observed across all amendments compared to non-amended soil, except for urea ($p < 0.05$) (Figure 20d). The abundance of AOA was higher in the compost (8.28 × 106 copies g-1 dry soil) compared to all other amendments. BC, BCU, and BCC amendments also showed an increase in AOA abundance relative to the non-amended soil. In contrast to AOA, AOB abundance was only

significantly higher in the compost amendment (2.69 × 107 copies g-1 dry soil) compared to other treatments (Figure 18e), suggesting a substantial stimulation of AOB communities by compost. The increase in AOB abundance has been reported in various studies, showing that AOB growth is only stimulated at high soil ammonia concentrations (Verhamme et al., 2011; Sterngren et al., 2015). In our study, compost and BCC initially had higher ammonium levels than other amendments, but these concentrations became negligible after four months of growth compared to nitrate concentrations.

The value of 2 for AOA to AOB ratio for the non-amended soil (Figure 18f) suggests a higher potential contribution of ammonia-oxidizing archaea to nitrification in the studied soil compared to ammonia-oxidizing bacteria. The results also indicate a decrease in the ratio of AOA to AOB in all amendments except BC in comparison to non-amended soil. Interestingly, only the BC amendment showed an elevated AOA to AOB ratio. This contrasts with most findings on other studies where it has been shown that Bacteria dominate microbial ammonia oxidation over Archaea in agricultural soil (Jia and Conrad, 2009). The differences in soil properties such as pH, N availability and other pedoclimatic conditions tend to affect the abundance and growth of AOA and AOB. For example, Sterngren et al., (2015) showed that AOA are important drivers of nitrification under N-poor conditions. In the same study, the addition of easily available N increased the abundance and relative importance of AOB in the nitrification process (Sterngren et al., 2015). This could explain the decrease in the ratio of AOA to AOB in compost, urea and their combination in comparison to non-amended soil. These amendments contain high levels of available N, particularly compost and urea. The correlation matrix indicated a positive correlation between AOB abundance and total mineral N in the soil as well as total cumulated mineral N leaching (Figure 2).

6.3.3 Genes abundances related to nitrogen denitrification processes

Similar trends were observed across these three genes nirK, nirS, nosZ abundances (Figure 18ghi), with the highest copy numbers generally found in the amendment with compost or BCC amendments, and the lowest in the soil without amendment. Higher copy numbers of nirK and nirS genes indicate an increased potential for denitrification, potentially leading to nitrogen loss via N2O emissions. However, compared with nirK, nirS appeared to be less abundant across all amendments. In addition, higher copy numbers of nosZ genes indicate an increased potential for complete denitrification, leading to a more efficient conversion of nitrate to N2. In the current study, all denitrification genes were positively correlated with total cumulated NO3- in leachates (Figure 2), which is the substrate for denitrifying enzymes. The increase in the denitrifying communities is commonly observed after the addition of compost or other organic amendments with high mineral available N pool (Maeda et al., 2011; Fu et al., 2020).

In line with our initial hypothesis, mixing compost with biochar decreased the abundance of AOA, nirK, nirS and nosZ in comparison to compost alone. Biochar has previously been identified as a potential amendment to reduce N2O emissions (Wang et al., 2013). These results are consistent with the finding of Cayuela et al., (2013), who demonstrated that biochar application decreased denitrification and consistently reduced N2O emissions by 10 to 90% across 14 different agricultural soils. The authors and other studies identified pH changes and N retention of biochar as the main factors influencing the effect of biochar on denitrifier communities (Cayuela et al., 2013; Pereira et al., 2015). In addition, Pereira et al., (2015) conducted a mesocosm study with silt loam soil cultivated with lettuce amended with wood biochar produced at different temperatures and found that the reduction in N2O emissions was

only observed with biochar produced at approximately 550°C. They suggested this effect may be transient, as decreases in N2O emissions were only observed for 3 days during the 42-day growing season and did not impact total N2O fluxes. In contrast, the application of biochar alone to soil had no significant effect on the abundance of denitrifying population, except nirS.

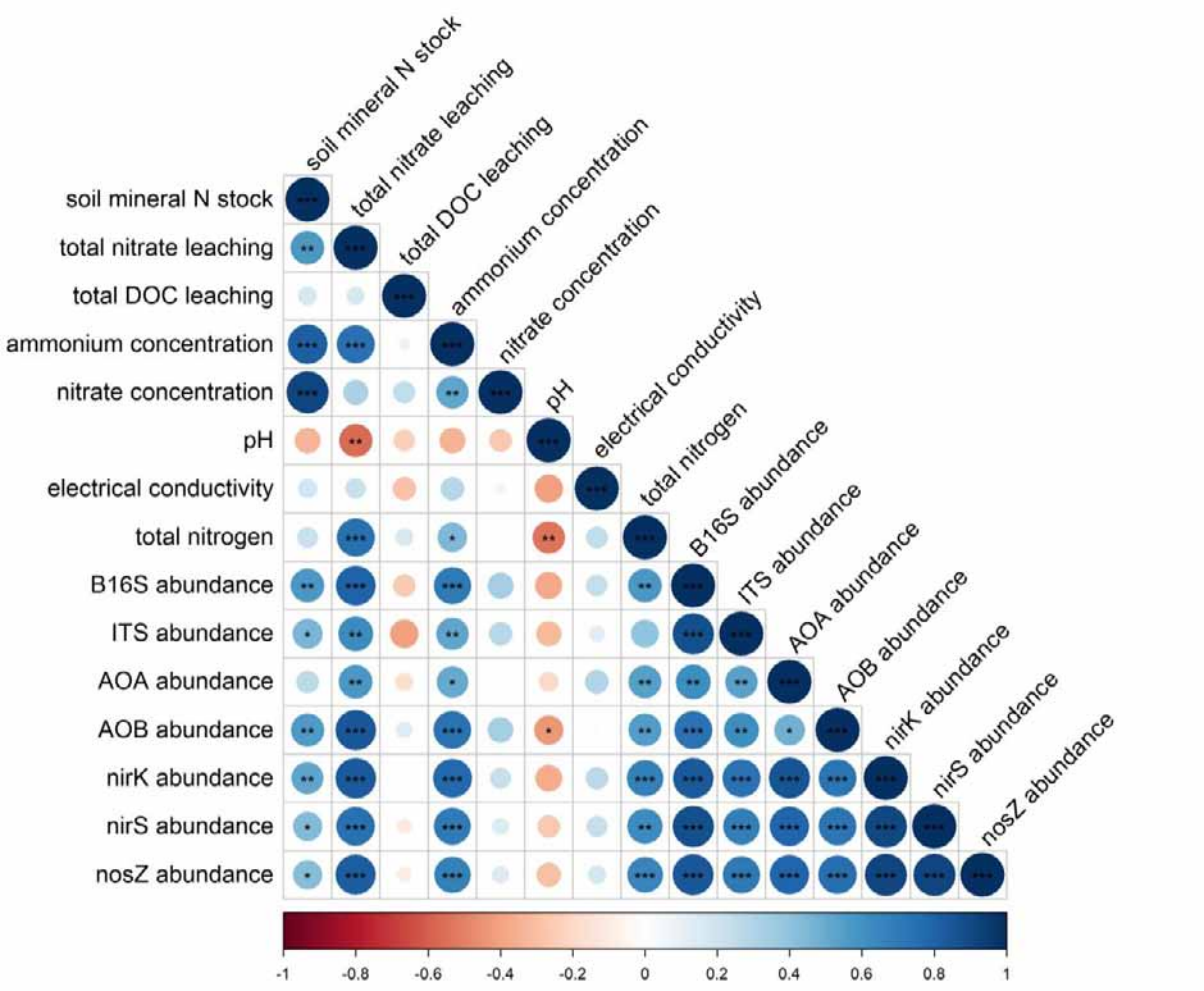

Figure 21 : Correlation matrix between N-cycling genes abundance and some soil parameters related to soil N dynamics. DOC: dissolved organic carbon. The size of the circles corresponds to the significance level ($p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$). Non-significant correlations are not shown.*

6.4 Conclusion

We studied the effects of various organic amendments derived from date palm residues, including biochar, compost, and their mixtures, on soil microbial community abundances involved in N-cycling in a sandy soil under an arid climate. Our findings showed that archaeal amoA adundance was higher in the non-amended soil, and that the addition of compost resulted in greater abundance of bacterial amoA in the mixtures containing compost. Compost amendment significantly increased the abundance of nirK, nirS, and nosZ denitrifier communities in comparison to non-amended soil, whereas the addition of biochar to compost reduced their abundance compared to compost alone. This may be due to the stabilization of organic matter related effects. Therefore, biochar amendment to sandy soil might contribute to reduce N loss in the form of N2O and N2 in arid agroecosystem. The interaction between biochar and compost should be further investigated to better understand its potential to optimize soil N management.

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Data statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

7 Conclusion

This study examines the effects of biochar and compost produced from date palm residues on soil improvement and microbial dynamics.

Biochar showed significant cation release (Ca, K, Mg, Na) when in contact with water, stabilizing after five rinses. Sodium adsorption by biochars was low (15–20%), regardless of oxygen content during pyrolysis. Even if biochar produced without oxygen (BC0) retained significantly less sodium, oxygen presence during pyrolysis had limited influence on adsorption specifically with high Na concentration in solution. Increased specific surface area with oxygen content did not significantly improve Na retention, suggesting that porosity and functional groups also play roles. Biochar's potential for mitigating soil salinization requires further exploration at varying temperatures.

Compost was evaluated from four mixtures: date palm residues with sheep manure (SM), poultry manure (PM), sewage sludge (S), and leachate (L). SM compost exhibited the best balance, with a reduced C_{org}/N ratio, high stability, and nutrient richness, making it ideal for sustainable agriculture. PM compost had high potassium content but low germination rates, indicating potential phytotoxicity. S compost showed maturity but lacked potassium, while L compost demonstrated slower degradation and stability. High electrical conductivity (EC) in all composts highlighted salinity concerns from organic matter mineralization and composting water.

Organic amendments used in field and pot experiments revealed biochar's high porosity and hydrophilic nature. Biochar's porosity (72.7%) increased significantly during pyrolysis, influencing adsorption and nutrient release dynamics. The compost from Palm compost company showed better stability compared to ASOC compost, which was less decomposed.

In microbial studies, compost significantly increased bacterial and fungal abundance, with low fungi-to-bacteria ratios due to soil texture and low organic carbon. Compost favored ammonia-oxidizing bacteria (AOB) and denitrifiers, enhancing nitrogen cycling, while biochar-compost mixtures mitigated N_2O emissions by altering pH and nitrogen retention. Biochar alone had minimal impact on denitrifiers, except for nirS gene abundance. These findings highlight biochar and compost’s complementary roles in soil health and nutrient management.

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Appendix 1

المعهد الوطني الجزائري للملكية الصناعية
INSTITUT NATIONAL ALGÉRIEN
DE LA PROPRIÉTÉ INDUSTRIELLE

الجمهورية الجزائرية الديمقراطية الشعبية
RÉPUBLIQUE ALGÉRIENNE
DÉMOCRATIQUE ET POPULAIRE

R2-FO-03
E1

Nature de la demande de protection *

Brevet d'invention ☑ | Extension de la demande internationale selon le PCT ☐ | Certificat d'addition ☐

[71] - **DEPOSANT[S]** : *Nom, Prénom, [dénomination], et Adresse complète*

Université Mohamed Khider Biskra
Faculté des sciences exactes et sciences de la nature et de la vie
Laboratoire diversité des écosystèmes et dynamique des systèmes de production agricole en zone arides (DEDSPAZA), B.P. 145, Biskra 07000

Nationalité du ou des déposants ALGERIENNE

[72] - **INVENTEUR[S]** : *Nom, Prénom, Adresse*

MASMOUDI Aya (1); GUIMEUR Kamal (2); MASMOUDI Ali (2); DEBILOU Abderrazak (3); SALHI Mouatez (4).
1- Laboratoire promotion de l'innovation en agriculture dans les régions arides (PIARA).
2- Laboratoire diversité des écosystèmes et dynamique des systèmes de production agricole en zone arides (DEDSPAZA)
3- Laboratoire d'Identification, commande, contrôle et communication (ECCC).
4- Ingénieur en électromécanique

[54] - **TITRE DE L'INVENTION** :

Pyrolyseur à gaz autonome et environnemental des déchets des végétaux.

[30] – **REVENDICATION DE PRIORITE (S)**

[31] - N°[s] de dépôt	[32] - date[s] :	[33] - pays d'origine	Nature de la demande

Numéro de dépôt	Date de dépôt	Heure
230164	22 FEV. 2023	14 : 11

N° de la demande internationale et date internationale de dépôt

Visa

BOUDJEMAI Sofia
Chef de Département
Service Dépôt
DTP

Demande de certificat d'addition rattaché au brevet principale n° **du**

[74] - **MANDATAIRE** : *Nom, Prénom, Adresse*

Date du pouvoir

Le préposé à la réception

Fait à : Alger le :

Signature et cachet
Qualité du signataire
pour les personnes morales

Autres informations

aya.masmoudi@univ-biskra.dz / 0676867736/0559476806
s.guimeur@univ-biskra.dz / 0696542950/0559378607
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BORDEREAU DES PIECES DEPOSEES *

- ☐ Copie de la demande internationale
- ☐ Mémoire descriptif en langue nationale
- ☐ Mémoire descriptif original en langue française
- ☐ Mémoire descriptif duplicata en langue française
- ☐ Dessin(s) original (aux) Planche(s)
- ☐ Dessin(s) duplicata (aux) Planche(s)
- ☐ Abrégé descriptif
- ☐ Pouvoir
- ☐ Document de priorité
- ☐ Cession de priorité
- ☐ Titre ou justification du paiement de taxes

Les demandes doivent être remises ou adressées par pli postal recommandé avec demande d'avis de réception, à l'Institut National Algérien de la Propriété Industrielle (INAPI) dont les coordonnées sont indiquées ci-dessous.

Le paiement des taxes exigibles peut être effectué soit directement auprès de la caisse de l'INAPI soit par virement bancaire au compte: BEA 12 Avenue AMIROUCHE, Alger- n° 00200012120326418071

Coordonnées de l'INAPI :

Adresse : 42, rue Larbi BEN M'HIDI, 3ème étage, B.P. 403 Alger Gare
Tél. : (021) 73 57 74 Fax: (021) 73 96 44 et (021) 73 55 81
E-mail: brevet@inapi.dz, info@inapi.dz - Web : www.inapi.dz

Le présent formulaire doit être lithographié

A NE PAS PLIER

* Cocher les cases correspondantes.

Appendix 2

R2-FO-03 E1DZ

الجمهورية الجزائرية الديمقراطية الشعبية
REPUBLIQUE ALGERIENNE DEMOCRATIQUE ET POPULAIRE

Demande N°:131

INSTITUT NATIONAL ALGERIEN
DE LA PROPRIETE INDUSTRIELLE

المعهد الوطني الجزائري للملكية الصناعية

REQUETE EN DELIVREANCE D'UN BREVET D'INVENTION
طلب منح براءة الإختراع

1 Natrure de la demande de protection — طبيعة الطلب

Brevet d'invention براءة الإختراع	Demande divisionnaire طلب جزئي	Certificat d'addition شهادة الإضافة
x		

Extension de la demande internationale PCT
الإمتداد عبر طلب دولي

2 INFORMATION SUR LE DEPOSANT — معلومات حول مقدم الطلب — 71

Dénomination: إسم الشركة: Université de Biskra

Forme juridique: الطبيعة القانونية: EPCA

Secteur d'activité: قطاع النشاط التجاري: service

Adresse: العنوان: BP 145 RP, 07000 Biskra | Wilaya: Biskra :الولاية | Commune: Biskra :البلدية

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RC: رقم السجل التجاري: 416020000070039

3 CODE DU MANDATAIRE — رمز الوكيل — 74

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4 INFORMATIONS SUR 'INVENTEUR — معلومات حول المخترع — 72

Nom et Prénom: الإسم واللقب: GUIMEUR Kamel

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5 DONNEES RELATIVES A LA PRIORITE — بيانات الأولوية — 30

Date: تاريخ: ////////// Numéro: رقم الأولوية: //////////

Pays d'origine: ////////// البلد الأصلي

CADRE RESERVER A L'INAPI
إطار خاص بالمعهد

BOUAKEZ El Hafsi
Chef de Service ZP

6 TITRE DE L'INVENTION — عنوان الإختراع — 54

Bioréacteur environnemental automatisé et paramétrable pour le compostage des déchets de palmiers

7 DOMAINE TECHNIQUE DE L'INVENTION — المجال التقني للإختراع — 51

//////////

8 DONNEES RELATIVES AU DEPOT — بيانات الإيداع

Date: تاريخ: 31 JUIL. 2023 Heure: الوقت:

Numéro: رقم الإيداع: 231389

9 DONNEES RELATIVES A LA DEMANDE INTERNATIONAL — بيانات الطلب الدولي

Date: تاريخ: Heure: الوقت:

Numéro: رقم:

10 DECHEANCE — إبطال

La déchéance d'un brevet d'invention intervient en cas de non-acquittement, à la date anniversaire du dépôt, des taxes de maintien en vigueur,

يسقط الحق على ملكية براءة الإختراع
في حالة عدم تسديد الرسوم السنوية المستحقة

SIGNATURE (CACHET)
ختم / توقيع

وزارة التعليم العالي والبحث العلمي
جامعة بسكرة
دبابش محمود

العنوان : ٤٢ شارع العربي بن مهيدي، الجزائر Adresse: 42, Rue Larbi BEN M'HIDI, Alger | E-mail: info-dpitt@inapi.org | Web : www.inapi.org

4	INFORMATIONS SUR L'INVENTEUR 2 معلومات حول المخترع	72
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Nationalité: الجنسية	Algerienne	
Adresse: العنوان	Université Mohamed Khider Biskra Faculté des sciences exactes et sciences de la nature et de la vie. Laboratoire diversité des écosystèmes et dynamique des systèmes de production agricole en zone arides (DEDSPAZA)	
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4	INFORMATIONS SUR L'INVENTEUR 3 معلومات حول المخترع	72
Nom et Prénom: الإسم واللقب	SEBIH Mahtali	
Nationalité: الجنسية	Algerienne	
Adresse: العنوان	Université Mohamed Khider Biskra Faculté des sciences exactes et sciences de la nature et de la vie. Laboratoire diversité des écosystèmes et dynamique des systèmes de production agricole en zone arides (DEDSPAZA)	
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4	INFORMATIONS SUR L'INVENTEUR 4 معلومات حول المخترع	72
Nom et Prénom: الإسم واللقب	BOUMARAF Belkacem	
Nationalité: الجنسية	Algerienne	
Adresse: العنوان	Université Mohamed Khider Biskra Faculté des sciences exactes et sciences de la nature et de la vie. Laboratoire promotion de l'innovation en agriculture dans les régions arides (PIARA)	
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4	INFORMATIONS SUR L'INVENTEUR 5 معلومات حول المخترع	72
Nom et Prénom: الإسم واللقب	MEHAOUA Mohamed Seghir	
Nationalité: الجنسية	Algerienne	
Adresse: العنوان	Université Mohamed Khider Biskra Faculté des sciences exactes et sciences de la nature et de la vie. Laboratoire Génétique, biotechnologie et valorisation de bio-ressources (GBVB)	
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4	INFORMATIONS SUR L'INVENTEUR 6 معلومات حول المخترع	72
Nom et Prénom: الإسم واللقب	DEBILOU Abderrazak	
Nationalité: الجنسية	Algerienne	
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4	INFORMATIONS SUR L'INVENTEUR 7 معلومات حول المخترع	72
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4	INFORMATIONS SUR L'INVENTEUR 8 معلومات حول المخترع	72
Nom et Prénom: الإسم واللقب	REBAI Messaouda	
Nationalité: الجنسية	Algerienne	
Adresse: العنوان	Université Mohamed Khider Biskra Faculté des sciences exactes et sciences de la nature et de la vie. Laboratoire diversité des écosystèmes et dynamique des systèmes de production agricole en zone arides (DEDSPAZA)	
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