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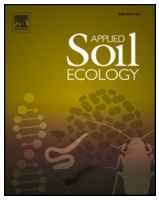

Influence of soil copper and zinc levels on the abundance of methanotrophic, nitrifying, and N_2O-reducing microorganisms in drylands worldwide

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ABSTRACT

Understanding soil microbial populations influencing biogeochemical cycles with potential implications for greenhouse gas (GHG) fluxes emissions is crucial. Methanotrophic, nitrifying and N_2O-reducing microorganisms are major drivers of CH_4 and N_2O fluxes in soils. The metabolism of these organisms relies on enzymes that require as cofactors metal ions scarcely available in the soil, such as copper (Cu) and zinc (Zn). Despite the importance of these ions, how their concentrations relate to the abundance of these microbes at the global scale has not been addressed yet. Here, we used data from a global survey carried out in 47 drylands from 12 countries to evaluate the role of soil Cu and Zn concentrations, and their relationship with aridity, as drivers of the abundance of methanotrophs, archaeal and bacterial nitrifiers, and N_2O reducers. To do so, we performed qPCR analyses of the marker genes *pmoA*, archaeal and bacterial *amoA* and *nosZI*. We did not find an association between the abundance of methanotrophs and Cu or Zn availability. However, our results highlight the importance of Cu influencing the abundance of nitrifying bacteria and N_2O reducers, two main actors involved in the N_2O cycle. Our findings indicate that dryland soils can be prone to reduce the N_2O coming from nitrification to innocuous N_2, but reductions in soil Cu availability (e.g., by increased aridity conditions due to climate change) could shift this trend.

1. Introduction

Microbial communities, because of their metabolism, play a pivotal role in the fluxes of GHG from soils (Dalal and Allen, 2008). Among these fluxes, methane (CH_4) and nitrous oxide (N_2O) are of great importance because of their high global warming potential (25 and 265 times higher than CO_2 over a 100-year period, respectively; IPCC, 2014). Although CH_4 is produced by methanogenic bacteria under anaerobic conditions, soils are generally considered a net sink for atmospheric CH_4 (Dutaur and Verchot, 2007). Under aerobic conditions, methanotrophic bacteria act as a sink of CH_4. These bacteria oxidize CH_4 through the activity of the particulate methane monooxygenase enzyme (pMMO), thereby contributing to the absorption of atmospheric CH_4. N_2O is released as a consequence of nitrifying and denitrifying activity, carried out by microorganisms that supply the soil with a variety of nitrogen (N) species in their oxidized and reduced forms. Ammonia-oxidizing archaea (AOA) and bacteria (AOB) initiate nitrification by oxidizing ammonia (NH_3) to hydroxylamine (NH_2OH) and then to nitrite (NO_2^-), which is finally converted to nitrate (NO_3^-) by nitrite-oxidizing bacteria. During nitrification, N_2O can be produced through NH_2OH oxidation and nitrifier denitrification (see Wrage-Mönnig et al., 2018, for a more in-depth review of these processes). NO_3^- is then reduced by denitrifying organisms to NO_2^- and, afterward, nitric oxide (NO), which is rapidly transformed to N_2O. Up to this point, denitrification is considered incomplete, as N_2O can be further reduced to molecular nitrogen (N_2). This final step can be executed by some denitrifiers, leading to complete

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denitrification, but also by organisms that harbor the so-called clade II or atypical N_2O-reductase enzyme. Nonetheless, N_2O reduction is not always performed and, thus, N can scape to the atmosphere in form of N_2O (see Hallin et al., 2018 for a more in-depth review about denitrification).

The abundance of soil microorganisms involved in CH_4 and N_2O fluxes is often constrained by the concentration of trace elements such as copper (Cu) and zinc (Zn), which act as essential enzymatic cofactors (Glass and Orphan, 2012). These influence methanotrophs because pMMO contains 9 Cu ions and 3 Zn ions (Nielsen et al., 1997; Trotsenko and Murrell, 2008; Semrau et al., 2010). Although some methanotrophs can express a non-copper dependent form, the soluble methane monooxygenase (sPMMO), under copper-deficient conditions (Semrau et al., 2018); pMMO is up to 30 % more efficient in CH_4 oxidation than sPMMO (Fru, 2011). Thus, Cu can potentially affect the capability of soils to absorb CH_4. However, environmental studies on the effects of Cu availability on methanotrophs are scarce (Glass and Orphan, 2012). Similarly, NH_3 oxidation and N_2O-reduction rates can vary depending on the concentration of Cu and Zn in the soil. Nitrifying bacteria and archaea carry out NH_3 oxidation to NH_2OH through the action of ammonia-oxidizing enzyme (AMO), which bacterial soluble form contains Fe, Cu and Zn (Gilch et al., 2009). While it is not clear whether Zn plays an active role in the activity of AMO and, therefore, is necessary for its functioning (Radniecki and Ely, 2008; Gilch et al., 2009; Corrochano-Monsalve et al., 2021), several studies have shown that there is a relationship between nitrification and the Cu concentration in the medium (He et al., 2018; Matse et al., 2022; Sereni et al., 2022). Soils with higher Cu concentration (but below toxic thresholds) would thus be prone to a greater abundance of nitrifiers and, in this manner, have a potential faster NH_3 oxidation and higher N_2O emissions. The reduction of N_2O to N_2 can be constrained by Cu because of the great needs of this element by the nitrous oxide reductase enzyme (N_2OR, Pomowski et al., 2011). Understanding the relationship of N_2O reducers with Cu is particularly important because this is the only known biological pathway able to act as a sink of N_2O, promoting the innocuous release of N to the environment in the form of N_2 (Thomson et al., 2012). Some studies in bacterial pure cultures (Sullivan et al., 2013; Black et al., 2016) and microcosms (Corrochano-Monsalve et al., 2021) have also shown a relationship between the abundance of N_2O reducers and the Cu concentration. In addition, denitrifiers seems to switch between a complete and an incomplete denitrification depending on the balance between NO_3^- and Cu in the medium (Van Spanning et al., 2007).

Despite its potential relevance, the role of Cu and Zn as drivers of methanotrophic, nitrifying and N_2O-reducing microorganisms across a wide range of environmental conditions has not been evaluated yet. Doing so in drylands is particularly interesting because they account for >40 % of the terrestrial surface and will likely expand during this century (Huang et al., 2016; Prăvălie, 2016). Since their metabolism requires aerobic conditions, both methanotrophic and nitrifying communities can have a relevant role in drylands. Despite drylands can be of special relevance as CH_4 sinks (Xu et al., 2022), studies about methanotrophy in these ecosystems are very scarce (Dutaur and Verchot, 2007). Similarly, and despite N_2O is released from dryland soils at low but constant fluxes during the dry season (Hu et al., 2017), the contribution of dryland soils to N emissions has been much less studied than that of more mesic biomes (Hu et al., 2017; Ramond et al., 2022). It has also been found that large N_2O emission episodes occur in dryland soils after rainfall (Hu et al., 2017; Krichels et al., 2022). In these "wetting-pulse" events, N that has been accumulated during the dry season due to the low biological activity typical of these ecosystems can be rapidly converted to oxidized and gaseous N forms by both nitrifying and denitrifying bacteria (Hu et al., 2017; Krichels et al., 2022). Considering the vast areas covered by drylands, the fluxes coming from these soils may play, indeed, an important role in the global N_2O emissions budget (Bowden, 1986). Similarly, N_2O-reduction remains understudied in arid zones and, therefore, we are unaware of the potential of these soils to release N intro the atmosphere in a less harmful form.

Here we used data from a global dryland survey (Maestre et al., 2012; Lafuente et al., 2019; Trivedi et al., 2019) to address how the abundance of microbes with Cu and Zn requirements might be influenced by the concentration of these trace elements in the soil and their relationship with aridity. To accomplish this goal, we quantified the abundance of the genes *pmoA*, *amoA*, and *nosZI*, widely acknowledged as indicators of methanotrophs, nitrifiers, and N_2O reducers, respectively. These microbial groups are considered main drivers of CH_4 and N_2O fluxes (Dalal and Allen, 2008). We hypothesize that the abundance of these microbes could be affected by the reductions in soil Cu and Zn availability observed as aridity increases across global drylands (Moreno-Jiménez et al., 2019). Moreover, we hypothesize that the presence of Cu can be more determinant for N_2O reduction in dryland soils than in other ecosystems because of their usually low NO_3^- content. This is so because under low NO_3^- availability there would be an energetic advantage on reducing N_2O to N_2 if enough Cu is available (Van Spanning et al., 2007; Felgate et al., 2012). In a world where atmospheric N_2O concentration is beginning to exceed the predicted levels across all scenarios (Tian et al., 2020), factors driving a shift in the potential capacity of the soil to produce and reduce N_2O might be of crucial importance for climate change (Schlesinger, 2009).

2. Materials and methods

This study was carried out in 47 dryland ecosystems from 12 countries (Argentina, Australia, Chile, China, Iran, Israel, Mexico, Morocco, Spain, Tunisia, United States and Venezuela) (Fig. 1). Locations covered a wide aridity gradient ranging from 0.5 to 0.94 (we calculated aridity as 1 – Aridity index, were aridity index is the mean precipitation/potential evapotranspiration), 67 to 766 mm yr^{-1} precipitation and from −1.8 to 22.4 °C mean annual temperatures. Samples were collected between 2006 and 2012 during the dry season, following a standardized protocol (Maestre et al., 2012, 2015). Briefly, a composite sample consisting of five 145 cm^3 soil cores (0–7.5 cm depth) was collected, bulked, and homogenized from five randomly selected bare ground areas (i.e., devoid of perennial vascular vegetation and located at least 1 m from plant canopies to minimize any effect of roots). After sieving the soil in the laboratory, one portion of each sample was air-dried for one month and stored for physicochemical analysis, while the other portion was frozen and stored at −20 °C for molecular analyses.

Our analysis was conducted on bare soils as they represent 64 % of the total surface area surveyed (ranging from 26 % to 97 % of coverage), thus being the most representative microsite across the sites studied. In addition, studying bare soils can provide a more accurate and controlled assessment when looking for general patterns (in this case, the effect of Cu and Zn on the abundance of methanotrophic, nitrifying and N_2O-reducing microorganisms). This is so for two main reasons. First, it is easier to separate the direct effects of Cu on these microbes from any indirect effect that might be mediated by complex plant-microbe interactions (Brunetto et al., 2016). Second, studying bare soils can reduce the potential confounding effects of plant species, root exudates or other factors that might vary among plant species. Plants can influence the availability of Cu and Zn in the soil by root uptake or exudation and thus, indirectly affect microbial abundance (Tao et al., 2004; Dotaniya and Meena, 2015). Nevertheless, if this were the case, it would be mediated by the same relationship between these trace elements and the abundance of microorganisms described below.

2.1. Soil properties

Soil pH was determined with a pH meter in a 1:2.5 (soil mass: water volume) suspension. Soils showed almost neutral to alkaline pH values, from 6.12 to 8.98. Total and available Cu and Zn contents were quantified from 0.5 g of soil by inductively coupled plasma atomic

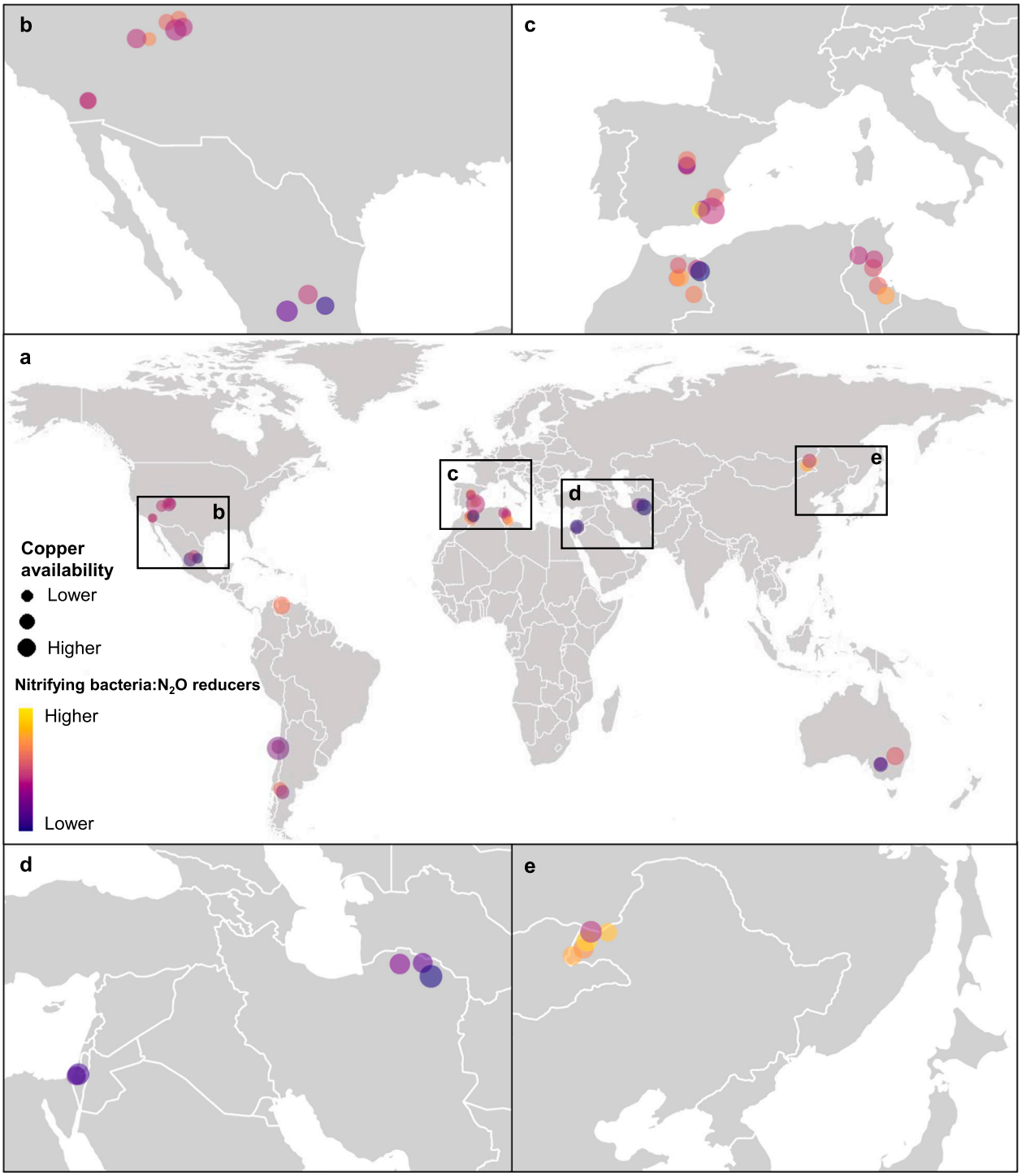

Fig. 1. Location of the 47 drylands sampled in this study differentiated by the availability of copper in the soil and the nitrifying bacteria: N_2O-reducers ratio. Global map (a) and closed-up maps of sited located in North America (b), the Mediterranean Basin (c), Middle East (d) and China (e). Map lines delineate study areas and do not necessarily depict accepted national boundaries.

spectroscopy (Iris Intrepid II XDL; Thermo Fisher Scientific) after digestion with HNO_3/H_2O_2 mixture and extraction with DTPA respectively, as described in Moreno-Jiménez et al. (2019). The samples presented total Cu concentration from 1.5 to 63.1 mg Cu kg dry soil^{-1}, while available Cu ranged from 0.08 to 3.9 mg Cu kg dry soil^{-1}. Total Zn concentration ranged from 3.1 to 82.4 mg Zn kg dry soil^{-1}, and available Zn from 0.08 to 4.37 mg Zn kg dry soil^{-1}. Other properties (soil texture, vegetation type, total and dissolved organic N, total P, and organic matter contents), can be consulted in Corrochano-Monsalve et al. (2024).

2.2. Molecular analyses

DNA was extracted from 0.5 g of frozen soil using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) following the manufacturer's protocol. Extracted DNA concentration and quality were determined by spectrophotometry with a NanoDrop® (ND-2000c, Thermo Scientific, MA, USA). To determine the abundance of

methanotrophs, nitrifiers, and N_2O reducers, quantitative polymerase chain reactions (qPCR) were performed using specific primers for the marker genes of these microorganisms: *pmoA* (pmo189f/pmo650r) (Bourne et al., 2001) for methanotrophs, archaeal *amoA* (CrenamoA23f/CrenamoA616r) and bacterial *amoA* (amoA-1F/amoA-2R) (Rotthauwe et al., 1997) for nitrifying archaea and bacteria, respectively, and *nosZI* (nosZ2f/nosZ2r) (Henry et al., 2006) for N_2O reducers. Each qPCR reaction was run in triplicate. Standard curves were prepared from serial dilutions of linearized plasmids with insertions of the target genes. The setting up for the reactions can be consulted in detail in Trivedi et al. (2019) (archaeal and bacterial *amoA*) and Lafuente et al. (2019) (*pmoA* and *nosZI*).

2.3. Statistical analyses

All data processing and statistical analysis were conducted in R version 4.1.2 (R Core Team, 2021) using Rstudio version 2022.02.0 (R Studio Team, 2016). We first checked the normality of all variables and transformed the data when necessary by applying the transformation that better fitted to a normal distribution according to the results obtained through the "bestNormalize" package in R (Peterson, 2021). We used Ordered Quantile (abundance of bacterial *amoA*, *nosZI*, *pmoA*, pH and available Zn), arcsinh (abundance of archaeal *amoA* and total Cu), Yeo-Johnson (available Cu), logarithmic (archaeal *amoA*: *nosZI* ratio), and boxcox (bacterial *amoA*: *nosZI* ratio) transformations.

Structural equation modelling (SEM) (Grace, 2006) was used to determine the effect of aridity and soil ions (Cu or Zn) as predictors of the abundance of methanotrophs, nitrifiers and N_2O reducers. Our a priori model included climate and soil properties as drivers of the abundance of these microorganisms (Supplemental Fig. S1). The abundance of methanotrophs and nitrifiers can depend on both Cu and Zn as both elements are present –or may be present– in the active site of their enzymes carrying CH_4 (pMMO) and NH_3 (AMO) oxidation (Gilch et al., 2009; Glass and Orphan, 2012). Thus, to analyze which ion could be a better predictor of their abundance, two models including Cu or Zn were addressed for methanotrophs and nitrifiers. In the case of N_2O reducers, only Cu was considered because Zn is not required for N_2O reduction (Pomowski et al., 2011). Nonetheless, since N_2O reducers could use not only available Cu, but also Cu bonded to ligands (Twining et al., 2007), two models were also fitted for these organisms, differing in the form of soil Cu considered (available or total). All variables were centered and scaled before SEM analysis to facilitate the interpretation of parameter estimates. To test the goodness-of-fit of the SEM model used, we used the Chi-square test and the Root Mean Square Error of Approximation (RMSEA); we assumed a good fit when $\chi^2 \leq 2$ and *P*-value >0.05, and when the RMSEA is close to 0 and *P*-value >0.05. We also verified that there was not spatial autocorrelation in our data by analyzing the distribution of residuals using the normality plots. These analyses suggested that spatial autocorrelation was not present in our data (Supplemental Fig. S4). All SEM analyses were conducted using the "lavaan" package version 0.6-11 (Rosseel, 2012).

3. Results

All genes analyzed presented a heterogeneous range of abundances across the drylands studied. *NosZI* showed the highest mean abundance (5.18×10^8 copies g dry soil^{-1}), followed by archaeal *amoA* (5.99×10^7 copies g dry soil^{-1}) and *pmoA* (4.67×10^7 copies g dry soil^{-1}), while bacterial *amoA* showed lower abundances (1.01×10^6 copies g dry soil^{-1}) (Table 1). Most soils were prone to present a greater abundance of *nosZI* genes than *amoA*, and in just three out of 47 sites we found more total *amoA* (archaeal + bacterial) than *nosZI* genes (Table 1).

Our SEM models including soil Cu as driver of the abundance of *pmoA*, archaeal *amoA*, bacterial *amoA*, and *nosZI* genes explained 4 %, 10 %, 21 % and 10 % of the variance, respectively (Fig. 2 and Supplementary Fig. S2). However, the relationship between Cu and the abundance of these genes was significant only in the case of bacterial *amoA* and *nosZI*. The abundance of bacterial *amoA* was negatively associated with Cu availability (path coefficient = −0.46; *P* = 0.004; Fig. 2b), while that of *nosZI* was positively related to total (path coefficient = 0.32; *P* = 0.038; Fig. 2c), but not to available (Supplemental Fig. S2c) Cu concentration. Although Cu is also necessary for AOA (as it is present in the structure of archaeal AMO, and even with a higher presence than in bacterial AMO, Glass and Orphan, 2012), there was no relationship between archaeal *amoA* and Cu content (Supplementary fig. S2a). Including Zn instead of Cu in the model improved its variance up to 18 % (Fig. 2a). Zn showed a negative relationship with the abundance of archaeal *amoA* that was, however, marginally significant (path coefficient = −0.32; *P* = 0.057). Similarly, we did not find an association of *pmoA* with neither Cu nor Zn, despite both microelements are cofactors of the pMMO enzyme (Supplementary Fig. S2e and f).

Table 1
Gene abundance, expressed as gene copy number per gram of dry soil, in global dryland soils, and ratios between gene abundances.

Gene abundance	Minimum	Maximum	Average	Median
Archaeal *amoA*	7.04×10^5	8.79×10^8	6.11×10^7	1.72×10^7
Bacterial *amoA*	1.07×10^5	5.61×10^6	1.03×10^6	3.40×10^5
nosZI	2.41×10^7	2.95×10^9	5.18×10^8	3.18×10^8
pmoA	8.35×10^5	2.45×10^8	4.67×10^7	2.06×10^7
Archaeal *amoA*: *nosZI*	1: 0.14	1: 1708	1: 129	1: 13
Bacterial amoA: *nosZI*	1: 21	1: 11030	1: 1372	1: 583
Total *amoA*: *nosZI*	1: 0.14	1: 1162	1: 98	1: 12

Aridity exerted a direct and negative impact on archaeal *amoA* (path coefficient = −0.32; *P* = 0.034) (Fig. 2a), but not on bacterial *amoA* (path coefficient = −0.28; *P* = 0.059) and *nosZI* (Fig. 2b and c). Nonetheless, our SEMs showed an indirect effect of aridity through its influence on soil pH, which was also indirectly associated with the abundances of these genes through a significant effect on soil Cu and Zn availability. Thus, soils from more arid zones exhibited a higher soil pH, which was associated with a lower Cu and Zn availability. In this manner, a greater aridity would exert a positive indirect effect on the abundance of archaeal and bacterial *amoA* genes, and a negative indirect effect on *nosZI*.

When analyzing the effect of the availability of Cu on the ratios between nitrifiers (which metabolism release N_2O) and N_2O reducers, our SEM models explained 13 % and 20 % of the variance of archaeal (Supplementary Fig. S2b) and bacterial (Fig. 2d) *amoA*: *nosZI* ratios, respectively. Nevertheless, the association of these ratios with soil microelements was significant only in the case of the bacterial *amoA*: *nosZI* ratio (path coefficient = −0.54; *P* = 0.001). The ratio between bacterial *amoA* and *nosZI* showed a negative relationship with soil Cu availability. In this case, we did not find a direct effect of aridity.

4. Discussion

4.1. The abundance of methanotrophs is not associated to copper or zinc concentration

Despite Cu and Zn are present in the structure of pMMO (Glass and Orphan, 2012), we did not find a significant association between the abundance of methanotrophs in global drylands and neither Cu nor Zn (Supplementary Fig. S2e and f). This agrees with the results obtained by Nazaries et al. (2018) in Scotland, who did not find any significant relationship between soil Cu concentration and methanotrophs. On the contrary, in a latitudinal gradient study of Chinese forest soils, Kou et al. (2021) observed both positive and negative relationships of methanotrophs with soil cations through the effects of pH on their availability. However, these effects differed between methanotrophic subgroups. These results suggest that different factors might exert distinct effects depending on the composition of the methanotrophic community and environmental conditions, making difficult to find global predictors of

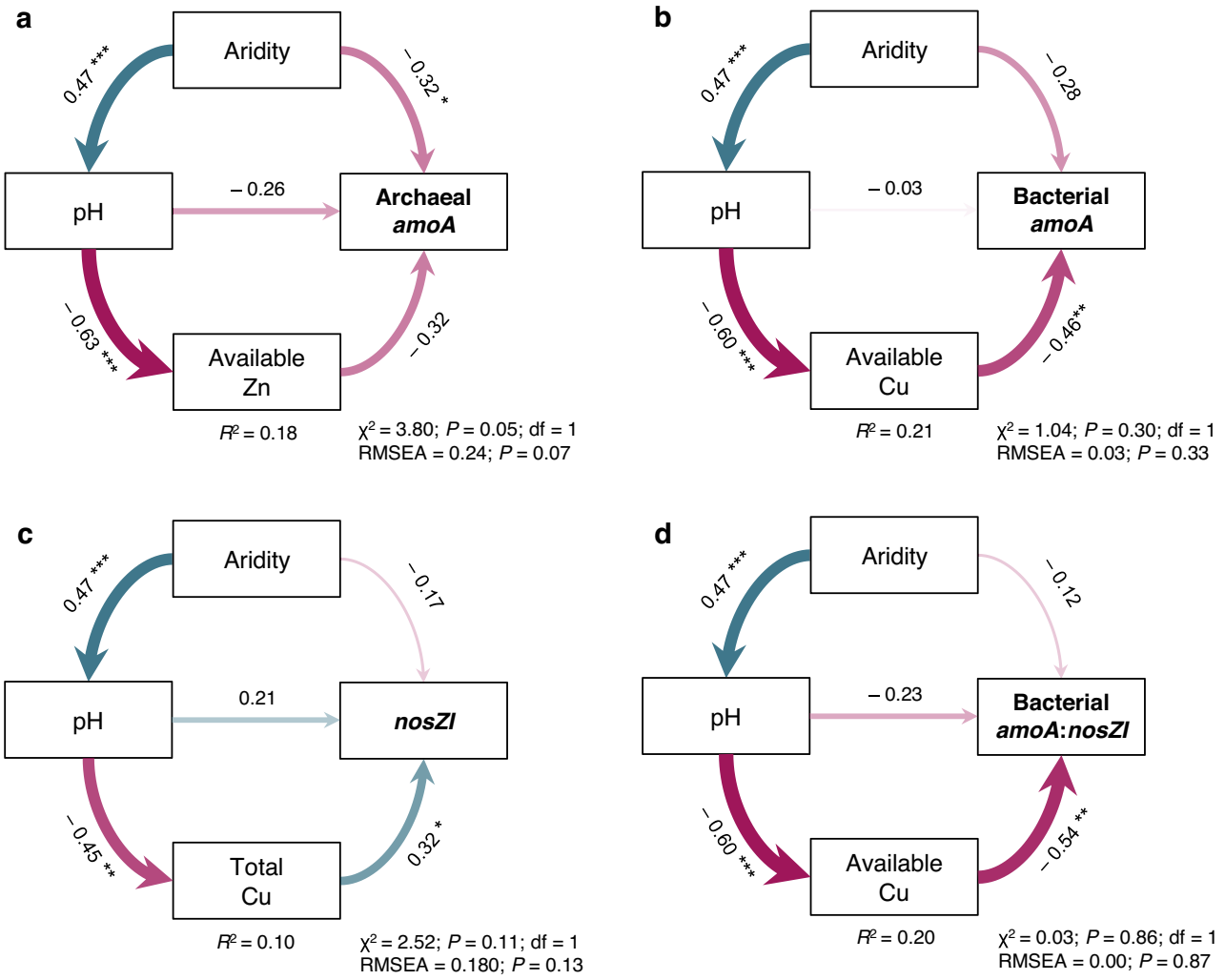

Fig. 2. Relationships between aridity, soil pH, soil Cu or Zn content and the abundance of nitrifying archaea (**a**; expressed as archaeal *amoA* abundance), nitrifying bacteria (**b**; expressed as bacterial *amoA* abundance), N_2O reducers (**c**, expressed as *nosZI* abundance) and nitrifying bacteria: N_2O-reducers ratio (**d**; expressed as respective genes abundances). The width of the arrows is proportional to the strength of the standardized path coefficients, which value is indicated adjacent to arrows. Blue and red arrows indicate positive and negative relationships respectively. The proportion of variance explained by the model appears below each gene. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

the abundance of these bacteria. This also seems supported by Lafuente et al. (2019), who did not find significant relationships between climatic and soil variables and the abundance of methanotrophs across global drylands.

In a laboratory experiment using paddy soils, Chang et al. (2021) concluded that methanotrophs might enhance N_2O emissions by competing for Cu with the N_2O-reducing community thanks to their ability for Cu scavenging. Our data showed an opposite trend in drylands, as we found a positive correlation between methanotrophs and N_2O reducers (Spearman, $\rho = 0.48$, $P < 0.001$). This seems to indicate that these populations were not under competition in the studied drylands and that laboratory studies carried out under particular environmental conditions cannot be generalized.

4.2. *Copper is linked to the abundance of nitrifying bacteria (but not archaea)*

Our SEM models indicated no relationship between soil Cu availability and the abundance of AOA (Supplementary Fig. S2a), suggesting that Cu might not be limiting for AOA growth in drylands. Interestingly, the variance explained by the model increased when Zn was included instead of Cu, although the negative relationship of Zn with the abundance of AOA was marginally significant ($P = 0.057$) (Fig. 2a). Nonetheless, we observed the same tendency as in other studies reporting that the AOA: AOB ratio decreased at higher Zn concentration due to a greater tolerance/adaptation of AOB community to excess of Zn (Mertens et al., 2009; Ruyters et al., 2010; Ruyters et al., 2013). In this manner, Zn seems to exert a negative effect on AOA at higher concentration, and might be a better predictor of the abundance of AOA than Cu. Interestingly, the effects of aridity on AOA were greater than those of Zn or Cu (Fig. 3a and Supplemental Fig. S3a) and opposite to the results reported by Delgado-Baquerizo et al. (2013, 2016). This is surprising considering the adaptation of AOA to limiting conditions, but might be related to the negative effect of a higher pH (associated to a greater aridity) on the abundance of AOA (Nicol et al., 2008). In this sense, our study might have captured this effect given the wider pH range of the analyzed sites in comparison those analyzed in Delgado-Baquerizo et al. (2013, 2016).

Contrary to our hypothesis, our results suggest that a greater Cu availability was associated with a smaller AOB community (Fig. 2b). This also contradicts previous in vitro studies demonstrating the stimulation of AOB growth by Cu (Ensign et al., 1993), which was also found using agricultural soils under laboratory conditions (Corrochano-Monsalve et al., 2021; Matse et al., 2022). We cannot assume that the results

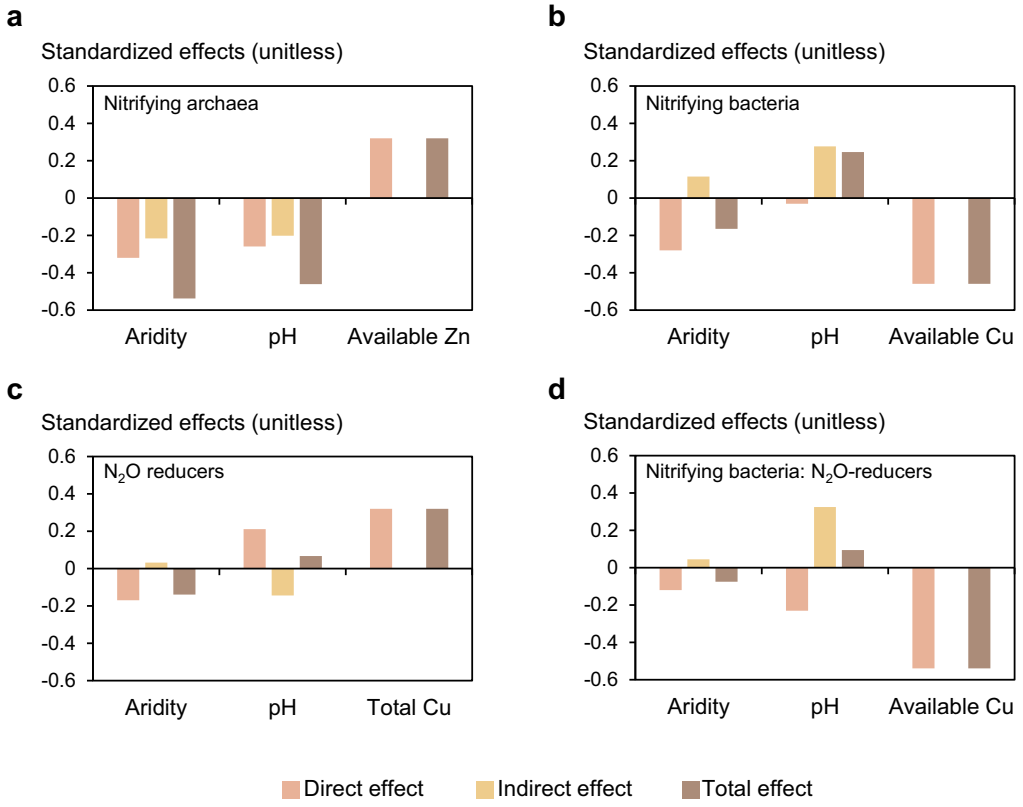

Fig. 3. Standardized direct, indirect, and total (direct plus indirect) effects of aridity, soil pH and Zn and Cu (total and available) contents on the abundance of nitrifying archaea (**a**) and bacteria (**b**), N_2O reducers (**c**) and the nitrifying bacteria: N_2O-reducers ratio (**d**). The effects presented are derived from the structural equation models shown in Fig. 2.

of these studies can be extrapolated to the distribution of these organisms across global drylands, but it is also difficult to explain that an enzyme harboring Cu as a cofactor presents a negative relationship with the availability of this element in the soil. Therefore, this should be analyzed to a greater depth in future experiments. The negative response of AOB might be related to other factors not captured in this study. Nonetheless, we could speculate with the existence of a threshold regulating the response of AOB to Cu in drylands through a hormetic response to Cu concentration (i.e., beneficial and toxic effects at low and high doses, respectively). Indeed, this has been observed in other ecosystems, in which Cu promotes nitrification when Cu concentration are low but inhibit it under high Cu concentrations (He et al., 2018; Matse et al., 2022; Sereni et al., 2022). Nevertheless, the Cu dose causing a negative effect on AOB seems to be very variable between these studies, from 3 to 1000 mg kg^{-1}. Furthermore, the tolerance to Cu might be lower in drylands than in other soils because: i) nitrification is more sensitive to Cu excess in soils with a prevailing low soil moisture history (Sereni et al., 2022), and ii) the low organic matter contents of dryland soils (Plaza et al., 2018), which would increase the exposition to Cu (Oorts, 2013). Thus, the Cu concentration in some of the dryland soils analyzed might be high enough for causing certain negative effects on AOB. In any case, our results indicate that an increase in aridity would promote the growth of AOB communities by indirectly decreasing the availability of Cu in dryland soils through an increase in soil pH. Soils with neutral and alkaline pH are metal-limited due to a greater bind of metals to organic and inorganic ligands (Mengel et al., 2001; Villaverde et al., 2009). Thus, aridity might boost the abundance of AOB –and possibly associated nitrification and N_2O production rates– by reducing AOB exposition to Cu.

4.3. *Copper concentration is linked to the abundance of N_2O reducers*

Denitrification occurs under anaerobic conditions. In consequence, the low water content of dryland soils during the dry season should be detrimental for N_2O-reducing bacteria. Nonetheless, these microorganisms can find anaerobic niches after wetting pulses and the greater microbial respiration rates that occur after these episodes (Parton et al., 1996). In fact, our data indicate that the abundance of N_2O reducers exceed that of total nitrifiers in drylands (Table 1). This indicates that the soils studied could be prone to reduce the N_2O coming from nitrification to N_2.

To our knowledge, only Lafuente et al. (2019) have analyzed how environmental factors, including aridity and soil properties like texture and pH, affect the distribution of N_2O reducers at the global scale. However, the role of soil Cu concentration as a possible driver of the abundance of N_2O reducers in natural ecosystems has not been studied yet, despite they require Cu for their metabolism (Pomowski et al., 2011). We found a positive relationship between Cu concentration and the abundance of N_2O reducers across drylands (Fig. 2c). This observation agrees with few previous studies analyzing the effect of Cu on N_2O reducers in bacterial cultures (Sullivan et al., 2013; Black et al., 2016) and soils under laboratory conditions (Corrochano-Monsalve et al., 2021). Nonetheless, our study provides the first empirical evidence of this relationship at the global scale and across a wide range of environmental conditions and total Cu contents (from 1.5 to 63.1 mg Cu kg dry $soil^{-1}$). Our results suggest that Cu limits N_2O reducers and these respond positively to increases in its concentration. They also suggest that forecasted increases in aridity (Huang et al., 2016; Prăvălie, 2016) may decrease the abundance of N_2O reducers and, thus, diminish the potential capability of drylands soils to reduce N_2O to N_2 (Figs. 2c and 3c).

4.4. Copper concentration influences the potential N_2O: N_2 emission ratio

Our results suggest that soil Cu concentration could be a main driver to regulate the form of N releasing in drylands and, thus, should be further considered to determine future scenarios evaluating N_2O emissions. Previous studies already suggested that soils with a greater Cu concentration might favor a more innocuous N loss (Richardson et al., 2009; Thomson et al., 2012; Shen et al., 2020). Although our model does not consider the effect of Cu on potential N_2O emissions from denitrification, it suggest that Cu promotes a potential lower N_2O: N_2 emission ratio when only potential N_2O emissions from nitrification are considered (Fig. 2d and Supplemental Fig. S2). Consequently, soils with lower Cu concentration can increase the AOB: N_2O-reducers ratio because a lower Cu concentration appeared favorable for AOB but unfavorable for N_2O-reducing bacteria. This may imply a greater N_2O: N_2 releasing ratio. Although our model agrees with studies indicating that the greater pH in arid soils directly favors a lower N_2O: N_2 ratio (Giles et al., 2012), the indirect effect of pH through Cu availability overpass this positive effect (Fig. 3d). Thus, our findings suggest that increases in aridity will reduce soil Cu concentration, and, potentially, may lead to enhanced release of N to the atmosphere in the form of N_2O coming from nitrification.

4.5. Concluding remarks

We conducted the first attempt to determine the importance of soil Cu and Zn concentration as determinants of the abundance of microorganisms involved in CH_4 and N_2O fluxes at the global scale. Our study presents only a limited frame: firstly, we only focus on the abundance of these microorganisms and, consequently, on the potential of these soils to carry out certain functions. Therefore, it would be necessary to confirm this potentiality by collecting data of CH_4 and N_2O emissions in these soils. Nonetheless, this would be a very logistically and economically costly approach, due to the difficulties of maintaining the setting-up and recurrent sampling in numerous and, in many cases, isolated areas around the world. Nonetheless, the abundance of these microorganisms has been successfully used for predicting GHG fluxes (Nazaries et al., 2013; Martins et al., 2015, 2017). Secondly, our study only focused on four of the groups involved in CH_4 and N_2O fluxes. Our purpose was just to determine the importance of soil Cu or Zn concentration as drivers of these specific microbes, but these preliminary results should be complemented by future studies considering additional groups of microorganisms. For instance, the relationship of Cu with both clade II (or atypical) N_2O reducers and incomplete denitrifiers carrying the enzyme nitrite reductase (the only enzyme harboring Cu within incomplete denitrification) should be addressed to complete our understanding. The relationships observed in this study should be also confirmed in other ecosystems, in particular those with low Cu availability (where the capability to reduce N_2O might be limited) and with additional Cu and/or Zn inputs, such as agricultural areas, to better understand GHG emissions by soil microbes involved in C and N cycling.

Our study represents the first attempt to assess the global significance of Cu concentration in soils as a driving factor for the prevalence of microbes implicated in GHG fluxes. Unexpectedly, we were not able to find an association between methanotrophic populations and Cu or Zn availability. On the other hand, soil Cu concentration was associated to bacterial nitrifiers and N_2O reducers. Thus, it could be a main factor determining the form and magnitude of N losses and the potential capability of soils to reduce N_2O to N_2. Even though our results indicate that dryland soils can be prone to reduce the N_2O coming from nitrification to N_2, an increase in aridity due to climate change could shift this tendency.

CRediT authorship contribution statement

Mario Corrochano-Monsalve: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Hugo Saiz:** Writing – review & editing, Writing – original draft, Supervision, Software, Methodology. **Fernando T. Maestre:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that support the findings of this study are openly available through "figshare" (Corrochano-Monsalve et al., 2024).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2024.105284.

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