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# N cycling increase after savanna afforestation with *Eucalyptus* or *Acacia* is reflected in the growth of soil ammonia-oxidizing archaea and nematode bacterial-feeders

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ABSTRACT

In tropical humid Africa, sandy soils under periodically burnt herbaceous savannas exhibit generally low carbon (C) content and nitrogen (N) availability. Savanna afforestation may overcome these limitations through changes in soil functioning, yet these processes still need to be explored. In this study, we investigated whether changes in the composition of soil micro-food web may explain soil C and N cycling increases following savanna afforestation. We conducted a 7-year experiment in Congo including *Eucalyptus* and $N_2$-fixing *Acacia* monocultures and *Eucalyptus-Acacia* mixtures established on former herbaceous savannas. We assessed in each of these modalities the soil attributes: organic C and N, $pH_{H2O}$, nitrate, ammonium, net C and N mineralization and nitrification rates, along with the abundances of bacteria, fungi, nematodes, ammonia-oxidizing archaea (AOA) and bacteria (AOB) in the top 10 cm layer. Afforestation of savannas with *Eucalyptus* for timber production increased soil C by 1.7 times, soil net N mineralization rates by 1.9 times and soil inorganic nitrogen by 2.5 times. Mixed *Acacia-Eucalyptus* and *Acacia* monoculture plantations further improved the rate of net nitrogen mineralization by a factor of 1.4 and soil inorganic N by a factor of 2.3 compared with *Eucalyptus* monocultures. These changes were associated with a gradual increase in AOA abundance from savanna to *Eucalyptus* monoculture, *Eucalyptus-Acacia* mixtures and finally *Acacia* monoculture. Savanna afforestation resulted in a significant increase in the absolute abundance of bacterial-feeding nematodes by 678 %, but to a decrease in the abundance of both fungal-feeders, and omnivores and predators. Increase in N cycling was positively associated with both AOA and nematode bacterial-feeder abundances, underlining the importance of monitoring micro-food web structure to understand better how land use changes affect soil biogeochemical cycling in the context of tropical afforestation.

## 1. Introduction

Tropical soils with low fertility such as Ferralic arenosols (FAO-UNESCO, 1994) are characterized by low organic matter content and cation exchange capacity (Mareschal et al., 2011), and rely mainly on plant litter decomposition by soil biota to function. However, in humid tropical Africa, herbaceous savannas – one of the major land uses on these soils – experience periodic fires that destroy their aerial biomass and result in N losses to the atmosphere (e.g., Jiang et al., 2022). However, savannas exhibit N conservation strategies in perennial grasses towards (i) strong internal N recycling within the vegetation – soil system – and more specifically towards and within the rhizosphere - and (ii) inhibition of archaeal nitrification leading to low soil N availability (Lata et al., 2022; Srikanthasamy et al., 2018; Abbadie et al.,

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1992). In southern Congo, part of these savanna soils has been afforested with fast-growing tree species since the 1970s, mainly *Eucalyptus* spp., for industrial production of pulpwood (Koutika et al., 2022). However, these plantations require the use of fertilizers to compensate for nutrient exports during each harvest, which has raised concerns about the environmental and economic sustainability of these systems (Bayle, 2019; Gonçalves et al., 2004). To address these issues, $N_2$-fixing tree species, particularly *Acacia* spp., have been introduced into *Eucalyptus* plantations in the recent decades. It had an overall positive effect on N balance, soil fertility as well as biomass production (Voigtlaender et al., 2019). However, the benefit of both savanna afforestation with *Eucalyptus* and the introduction of *Acacia* into areas where *Eucalyptus* had been grown as a monoculture for many years on soil fertility is highly variable, depending mostly on the pedoclimatic context, plantation age and management practices (Pereira et al., 2024; Morazzo et al., 2021; Tchichelle et al., 2017).

Changes in soil functioning following savanna afforestation have rarely been linked to the soil micro-food web structure. Increasing N inputs with *Acacia* introduction has mostly focused on the study of functional genes such as the bacterial and archaeal ammonia monooxygenase-alpha subunit (*amoA*), bacterial nitrite reductase gene (*nirK*) and bacterial nitrogenase reductase (*nifH*) (Pereira et al., 2019; Rachid et al., 2013), or the composition of bacterial and/or fungal communities (Pereira et al., 2021; Koutika et al., 2020; Rachid et al., 2015). However, the divergent responses of these communities to *Acacia* introduction make difficult to establish a generic pattern. This issue is a common challenge when attempting to connect microbial community composition with ecosystem functions, due to analytical and statistical limitations (e.g. Bier et al., 2015), coupled with the high level of functional redundancy within this community (Louca et al., 2018; Nielsen et al., 2011).

A more consistent pattern emerged when considering the relative proportion of the bacterial and fungal energy channels, the former being considered to promote more active N cycling through acquisitive strategies, while the latter is regarded as more conservative in both N and C cycles (de Vries and Bardgett, 2012; de Boer et al., 2005; Wardle et al., 2004). This approach can be applied not only to the microbial community but also through the abundance of their respective grazers and, more broadly, to the composition of soil food web (e.g. de Vries et al., 2013) through their positive feedbacks on nutrient cycling. Top-down regulation affects not only microbial community composition but also their activities, with regard to nutrient cycling (Trap et al., 2016; Blanc et al., 2006). Consequently, the structure of the soil micro-food web – i.e. the fast energy channeling size-structured component of the below-ground food web, composed of bacteria and fungi as basal resources and protists and nematodes as primary fauna consumers (Potapov et al., 2021) – may sometimes be more easily linked with soil functions than the microbial community itself (Sauvadet et al., 2016; A'Bear et al., 2014; de Vries et al., 2013). The importance of microbial grazing on N cycling was further supported with inoculation experiments of bacterial-grazer nematodes, in temperate soil (Brondani et al., 2022) and tropical, nutrient-poor ferrallitic soil (Trap et al., 2021; Ranoarisoa et al., 2018). In particular, Trap et al. (2021) underlined the potential of supporting free-living nematode abundance and activity through plant community diversification to increase soil fertility, especially in agroecosystems on nutrient-depleted soils. However, to our knowledge, this potential was not assessed in tropical tree plantations, despite promising results from legume tree associations with coffee plants on Acrisols and Cambisols (Sauvadet et al., 2019).

Our objective was to determine whether changes in soil C and N cycling – from savanna to *Eucalyptus* plantations, and then planting *Acacia* trees after *Eucalyptus* monocultures – were associated with soil micro-food web differentiation. We worked in a 7-year-old experiment set up on former *Eucalyptus* monocultures (and previously on savanna) comparing *Eucalyptus* monocultures, *Acacia* monocultures and mixed 50:50 *Eucalyptus*–*Acacia* plantations. We hypothesized that (i) savanna afforestation would lead to higher microbial-feeder abundance and greater top-down regulation (inferred as nematode predators and omnivores abundance), regardless of tree species, which will subsequently increase C and N cycling and that (ii) increased N inputs with *Acacia* introduction would lead to the promotion of AOA and the decrease in the fungal: bacterial energy channel ratio, that will both exert positive feedbacks on N cycling.

## 2. Material and methods

### 2.1. Site description

The study site is located on the Atlantic coast near Pointe Noire city, Kissoko village, Congo (4°44′ S & 12°01′W), on a Ferralic Arenosol with a sandy texture (Laclau et al., 2010). The soil properties are described in detail in Waithaisong et al. (2020). The climate is humid tropical with a dry season (June to September) according to Köplen and Geiger classification. The native ecosystem is a tropical savanna (S), dominated by Poaceae species (Epron et al., 2013). It was then afforested in 1984 with the hybrid of *Eucalyptus grandis* W.Hill ex Maïden and *Eucalyptus urophylla* S.T. Blake (*E. urophylla* x *grandis*) for pulpwood production, and knew three 7-years rotations before the experimental trial set up in 2004. This set up consisted in the introduction of *Acacia mangium* Willd, an exotic $N_2$-fixing legume tree, to compare wood production under different silvicultural practices.

The experimental trial involved seven plantation modalities (see Bouillet et al., 2013 for more details) including the establishment of *E. urophylla* × *grandis* (E) monocultures, *A. mangium* monoculture (A), and mixed *Acacia-Eucalyptus* plantation at a proportion of 50:50 (AE). Trees within these three plantation modalities (both *Eucalyptus* and *Acacia*) were fertilized at planting in May 2004 with 43 g N plant$^{-1}$ (34.4 kg N ha$^{-1}$) within a radius of 50 cm around tree trunks (Bouillet et al., 2013). The experimental site included five blocks of over 7000 m$^2$ including these three plantation modalities, which were separated with at least two tree rows to avoid border effect. Individual plots – corresponding to a given plantation modality – were randomized within each block. They were made of 6 rows of 6 trees (inner rows) with two additional surrounding buffer rows, with a stocking density of 800 trees ha$^{-1}$ and a total area of 1011 m$^2$.Of the five blocks, one was used for destructive sampling, and another suffered high mortality rates in *Acacia* monoculture. Hence, soil sampling was performed on the three remaining blocks, for all the modalities studied.

### 2.2. Soil sampling

Soil sampling was carried out with an auger in the 0–10 cm layer at the end of the rainy season, 7 years after plantation establishment, in three blocks for each planting modality, giving nine plantation plots. Nearby savanna (about 200 m from the site) with similar physical soil properties and topography as the plantation experimental site, was selected. The following sampling design was adopted for savanna, in order to be similar to the strategy used in the plantations. First, three nearby locations were chosen in the savanna, at a distance alike to the one between blocks in the plantation experimental site. Then, each of the three location was sampled along a 10-m transect where a soil core was taken every meter, to obtain one composite sample per location, and 3 replicated composite soil samples for savanna land use (see Waithaisong et al., 2020 for more detail). For each *Eucalyptus* and *Acacia* monoculture plot, 3 pairs of trees were selected along a diagonal near the center of the plot, which were subsequently sampled following the Voronoï square method to create one composite soil sample per pair of trees, and thus 3 pseudo-replicates per plot. The same strategy was doubled in *Eucalyptus* – *Acacia* mixtures plots to consider the influence of *Eucalyptus* and *Acacia* individuals: 3 pairs of *Eucalyptus* trees were thus chosen to assemble 3 composite samples close to *Eucalyptus* individuals (the E/AE modality), in parallel with 3 pairs of *Acacia* trees to assemble

3 composite samples close to *Acacia* individuals (the A/AE modality). Each soil composite sample was subsequently thoroughly mixed, sieved at 2 mm, then split in various aliquots for the different analyses. Fresh soil was used for assessment of inorganic N content (i.e. nitrate and ammonium contents), net C and N mineralization, nitrification and nematode communities. Subsamples were also kept at −20 °C for subsequent molecular analysis. Another aliquot was air-dried, which was used for pH ($H_2O$) measurement, while another aliquot was ground to 0.2 mm for soil organic C and N determination.

### 2.3. *Soil biochemical analyses*

Total soil organic C and N were determined by dry combustion of soil samples ground to 0.2 mm, using a CHN micro-analyzer (Carlo Erba NA 2000). Soil $pH_{H20}$ was determined by mixing 2 g of dry soil with 10 ml of deionized water for 30 min (ISO 10390:2005). Soil net nitrification, C and N mineralization rates were measured according to an adaptation of the method described in Isaac et al. (2011) and Waithaisong et al. (2023). First, initial soil inorganic N was extracted from 10 g of fresh soil, with 50 ml of a 1 M KCl solution. $NO_3^-$ and $NH_4^+$ were determined by continuous flow colorimetry (TRAACS 2000, Bran and Luebbe, Norderstedt, Germany), as described by Krom (1980) and Kamphake et al. (1967), respectively. Then, an aliquot of 25 g fresh soil was put in a sealed jar after fixing its water content at a potential of pF 2.5. Jars were then incubated at 28 °C for 28 days with an alkali trap (15 ml of 0.5 M NaOH). The traps were changed after 7, 14 and 28 days and analyzed for carbonates within the day. The remaining NaOH was titrated with 1 M HCl. The final soil inorganic N content was assessed after 28 days of incubation of the 25 g jars. Net C and N mineralization rates were deduced from the amount of $CO_2$, and inorganic N ($NO_3^-$ and $NH_4^+$) produced during this 28-day incubation, respectively.

### 2.4. *Soil microbial analyses*

Total soil genomic DNA was extracted from 125 mg of soil by using the "FastDNA SPINTM kit for soil" (MP Biomedicals Santa Ana, CA, USA) as described by Tournier et al. (2015). Quantification of total extracted DNA was done using PicoGreen fluorescence (Molecular Probes, Paris, France) with SYBR Green as fluorescent dye measured on a BioRad Thermocycler C1000 CFX-96 (BioRad, Hercules, CA, USA). Final elutions were kept at −20 °C until amplification.

Quantitative PCR (qPCR) was used to quantify gene copy numbers of bacteria, fungi, AOA and AOB communities. First, DNA extracts were diluted to a final concentration of 1.25 ng. $\mu l^{-1}$ for each soil sample. 4 replicates out of the 9 sampled per modality were then chosen randomly and were analyzed in qPCR. The reduction of repetitions made possible to analyze all the samples on a same plate to limit the variability due to variations between plates. qPCR assays were run in plate duplicate using a Biorad CFX96 Real-time PCR System with a SYBRGreen detection and the results were calculated as mean values of these assays. Details on the primers used as well as qPCR conditions are given in Table S1 (Supplementary data). 2.5 ng DNA were amplified in a total reaction volume of 10 μl, containing 1× of absolute QPCR SYBRGreen mix ® (Thermoscientific, AB-1159/A) and 0.5 μM of each primer. Two no-template controls were also included in all the assays. Standard curves were obtained using tenfold serial dilutions of a linearized plasmid pGEM-T Easy Vector ($10^2$ to $10^8$ copies according to the gene) containing the targeted gene for all qPCR assays. The reliability of the standard curves was controlled by verifying reproducibility of the Ct values, the quality of the dilutions series and ensuring that the $r^2$ was higher than 0.98. To verify the specificity of the primers, melting curves were generated after amplification by increasing the temperature from 55 °C to 95 °C (+0.5 °C per increment). Purity of the amplified products was checked by observation of a single melting peak. The qPCR results were analyzed with the Software Bio-Rad CFX Manager 2.0.

### 2.5. *Soil nematode community analyses*

Nematodes were extracted from 200 g soil samples (fresh weight) by elutriation with cotton–wool filter method (Seinhorst, 1962; Townshend, 1963) and counted under binocular microscope. After fixation in formaldehyde–glycerol (4 %), 100–150 specimens per sample were randomly selected in mass slides and identified to family and genus levels under a microscope. The taxa were then assigned to a trophic group (Yeates et al., 1993) and a colonizer-persister (cp) class (Bongers, 1990), which rates the taxa life strategy from extreme r- (cp1) to extreme K-strategists (cp5), which allowed to assign to each taxa a functional guild (Bongers and Bongers, 1998). As the abundance of the functional guilds studied were highly correlated with the classical nematode based indices (Structure Index, Basal Index, Enrichment Index…) in our study, we chose to present only data of group abundance in this article.

### 2.6. *Data analyses*

One sample (modality A-AE, block 2, first pseudo-replicate) could not be extracted for nematofauna, and was hence removed from the dataset. The significance of the land use impacts on each variable measured was assessed with general linear mixed-effects models, with the block identity as random effects for variables which could meet the conditions of normality and homoscedasticity. For variables that were not normal, Kruskall-Wallis tests followed with Wilcoxon rank comparisons were used. Redundancy analyses (RDA) were then performed on soil biogeochemical properties constrained by soil communities' indicators, followed by a Pearson correlation matrix on the dataset with correlations corrected for multiple comparisons with the Benjamini-Hochberg method. All statistical analyses were performed using R software (R-4.3.1) and the following packages: car (Fox and Weisberg, 2019), ggplot2 (Wickham, 2016) lme4 (Bates et al., 2015), lsmeans (Lenth, 2016), multcomp (Hothorn et al., 2008), psych (Revelle, 2024), reshape2 (Wickham, 2007), stats (R Core Team, 2023) and vegan (Oksanen et al., 2024).

## 3. Results

### 3.1. *Soil properties, biological activities and communities*

Savanna afforestation increased greatly soil C content (+ 60 %: + 4 g C $kg^{-1}$ soil), C:N ratio (+ 34 %), and decreased soil $pH_{H2O}$ (− 13 %), regardless of plantation types (*P*-values <0.05, Table 1). However, savanna afforestation did not impact on soil C mineralization rates (P-values >0.05). Afforestation improved N cycling indicators, with varying amplitude depending on plantation types. This increase was moderate in the case of afforestation with *Eucalyptus* monoculture (3.4 and 1.9-fold increase of nitrification and net N mineralization rates), and stronger when savanna was afforested with *Eucalyptus* – *Acacia* mixtures, independently of the sampling position within these plantations (7.4 and 2.9-fold increase of nitrification and net N mineralization rates). Afforestation with *Acacia* monoculture allowed notably to increase nitrification rates compared with the *Eucalyptus* – *Acacia* mixtures, but not net N mineralization rates, which remained at the same levels than those found in the mixed plantations (Table 1). Overall, soil nitrate and inorganic N contents also increased gradually from savanna, *Eucalyptus* monocultures, *Eucalyptus-Acacia* mixtures and *Acacia* monoculture, but remained low under all the modalities (inferior to 5.5 mg N $kg^{-1}$ dry soil, Table 1) (Recous et al., 1995).

Savanna afforestation led to great changes in nematode community composition, which were similar between plantation types (Table 2). First, the abundances of bacterial-feeders cp1 and cp2–4 were 37 and 7 times higher respectively in the plantations than in the savannah. Second, the abundances of fungal- feeders, omnivores and predators were respectively around 3 and 8 times higher in the savannah than in the

**Table 1**

Soil properties and potential mineralization rates on the 0–10 cm layer. For each variable, significant differences ($p$-values <0.05) were tested using general linear mixed-effects models, with land use as fixed effect and block identity as random effect, and are indicated by different letters. E: eucalyptus monoculture; E-AE: under eucalyptus trees in mixed plantation; A-AE, under acacia trees in mixed plantation; A: acacia monoculture; S: herbaceous savanna.

| | Savanna | Eucalyptus | Mixed Acacia-Eucalyptus | | Acacia |
|---|---|---|---|---|---|
| | S | E | E-AE | A-AE | A |
| Soil C *(%)* | 0.64 ± 0.04 c | 1.06 ± 0.22 a | 1.08 ± 0.10 a | 1.04 ± 0.14 ab | 0.90 ± 0.12 b |
| Soil N *(%)* | 0.04 ± 0.00 b | 0.05 ± 0.00 a | 0.05 ± 0.01 a | 0.05 ± 0.00 a | 0.05 ± 0.01 a |
| Soil C:N | 14.3 ± 1.1 c | 20.1 ± 2.7 a | 20.9 ± 1.9 a | 19.4 ± 1.8 ab | 18.8 ± 2.1 b |
| Nitrate *(mg kg$^{-1}$)* | 0.07 ± 0.03 c | 0.41 ± 0.35 c | 1.95 ± 0.81 b | 2.12 ± 0.78 b | 3.69 ± 0.99 a |
| Inorganic N *(mg kg$^{-1}$)* | 0.73 ± 0.20 d | 1.84 ± 0.42 c | 3.58 ± 1.20 b | 3.76 ± 1.14 ab | 5.29 ± 1.63 a |
| pH ($H_2O$) | 5.35 ± 0.17 a | 4.69 ± 0.20 b | 4.76 ± 0.12 b | 4.64 ± 0.16 b | 4.62 ± 0.17 b |
| C mineralization *(mg kg$^{-1}$ d$^{-1}$)* | 5.77 ± 1.64 ab | 7.57 ± 4.10 a | 5.90 ± 2.40 ab | 4.96 ± 2.06 ab | 4.57 ± 2.10 b |
| N mineralization *(mg kg$^{-1}$ d$^{-1}$)* | 0.14 ± 0.03 c | 0.26 ± 0.05 b | 0.41 ± 0.07 a | 0.37 ± 0.07 a | 0.34 ± 0.06 a |
| Nitrification *(mg kg$^{-1}$ d$^{-1}$)*° | 0.03 ± 0.20 d | 0.09 ± 0.06 c | 0.19 ± 0.06 b | 0.20 ± 0.03 b | 0.29 ± 0.05 a |

Data are the average ± the standard deviation of the 9 observations for each land use.

° indicates a test of kruskall-wallis coupled with Wilcoxon rank sum test because of the non-normality of the variable.

**Table 2**

Abundance and taxonomic composition of soil microbial and nematode communities on the 0–10 cm layer. For each variable, significant differences (p-values <0.05) were tested using general linear mixed-effects models, with land use as fixed effect and block identity as random effect, and are indicated by different letters). E: eucalyptus monoculture; E-AE: under eucalyptus trees in mixed plantation; A-AE, under acacia trees in mixed plantation; A: acacia monoculture; S: herbaceous savanna. NCR: ratio between bacterial-feeders and the sum of bacterial-feeders and fungal-feeders.

| | Savanna | Eucalyptus | Mixed Acacia-Eucalyptus | | Acacia |
|---|---|---|---|---|---|
| | S | E | E-AE | A-AE | A |
| Microbial community abundance *(log gene copies g$^{-1}$ dry soil)* | | | | | |
| Bacteria | 9.02 ± 0.06 a | 9.00 ± 0.08 a | 9.02 ± 0.09 a | 8.95 ± 0.08 ab | 8.88 ± 0.10 b |
| Fungi | 7.62 ± 0.15 ab | 7.73 ± 0.14 ab | 7.70 ± 0.12 ab | 7.58 ± 0.15 b | 7.81 ± 0.29 a |
| Fungi: Bacteria ratio | 0.04 ± 0.01 b | 0.06 ± 0.02 ab | 0.05 ± 0.02 b | 0.05 ± 0.02 b | 0.10 ± 0.07 a |
| Ammonia oxidizers | 6.29 ± 0.51 c | 6.81 ± 0.31 b | 7.26 ± 0.16 a | 7.21 ± 0.08 a | 7.26 ± 0.13 a |
| Nematodes community abundance *(individuals 100 g$^{-1}$ dry soil)* | | | | | |
| cp1 Bacterial-feeders (Ba1) | 2 ± 3 b | 83 ± 139 a | 60 ± 67 a | 78 ± 47 a | 145 ± 100 a |
| cp2–4 Bacterial-feeders (Ba2–4) | 62 ± 17 b | 420 ± 286 a | 404 ± 213 a | 484 ± 222 a | 312 ± 162 a |
| Fungal-feeders | 88 ± 48 a | 48 ± 30 ab | 31 ± 28 b | 33 ± 33 b | 18 ± 6 b |
| Omnivores & predators | 71 ± 20 a | 9 ± 6 b | 5 ± 5 b | 9 ± 7 b | 11 ± 22 b |
| Total free-living | 223 ± 64 b | 561 ± 339 a | 502 ± 232 a | 605 ± 251 a | 486 ± 172 a |
| Nematode Channel Ratio NCR° | 0.44 ± 0.17 b | 0.89 ± 0.10 a | 0.94 ± 0.04 a | 0.95 ± 0.03 a | 0.96 ± 0.02 a |

Data are the average ± the standard deviation of the 9 observations for each land use.

° indicates a test of kruskall-wallis coupled with Wilcoxon rank sum test because of the non-normality of the variable.

plantations (Table 2). Although not significant, slight differences could be observed between *Acacia* monoculture and the other plantations, with on average higher abundances of cp1 bacterial-feeders but lower abundances of fungal-feeders. On the other hand, the abundances of bacteria and fungi were not affected by savanna afforestation ($P$-values >0.05), with the exception of a slight decrease of bacteria abundance under *Acacia* monocultures (Table 2). Finally, afforestation increased the abundance of AOAs, but to a variable extent depending on the type of plantation, since afforestation of savanna with *Eucalyptus* monoculture increased the number of AOA gene copies by a factor of 2.5, whereas afforestation with *Eucalyptus-Acacia* mixture or *Acacia* monoculture increased this number by a factor of 5.6.

### 3.2. Relationships between soil properties and communities

The first axis of the redundancy analysis performed across all land uses explained 44.3 % of the variance (Fig. 1a). On this axis, soil nitrate and inorganic N, nitrification and net N mineralization rates all positively covariated with AOA and cp1 bacterial-feeders abundances. These variables covariated negatively with the abundance of fungal-feeders, omnivores and predators, and soil pH$_{H2O}$ on the same axis. In contrast, the abundance of cp2–4 bacterial feeders exhibited orthogonality with the indicators for the other communities and was positively associated with soil organic C and N contents (Fig. 1.a). The distribution of the sites on the factorial plan was prominent along the first axis of the redundancy analysis, with the particular positioning of savanna sites, associated with higher soil pH values, omnivores-predators and fungal-feeders. The plantation sites were associated with higher soil nitrate, inorganic N, C and N contents, net N mineralization and nitrification rates, along with greater AOA and the abundance of bacterial-feeder nematodes (Fig. 1a). The marked contrast between savannas and plantations drove the relationships between the soil and community indicators, with slight variations when the RDA focused exclusively on the plantation sites (Fig. 1b). Nitrate and soil inorganic N remained positively associated with both AOA and cp1 bacterial-feeders abundance. However, soil C and N content, pH$_{H2O}$ and net C mineralization rates covaried positively with cp2–4 bacterial-feeders, fungal-feeders and bacteria abundances in the RDA1 axis. On the other hand, the plantations with *Acacia* differentiated mostly on the RDA1 axis, with an impoverishment in fungal-feeders and soil C content to the benefits of nitrate, inorganic N and AOA towards acacia mixtures then monocultures (Fig. 1b).

Pearson correlation matrices highlighted further the relationships between soil micro-food web components with soil biogeochemical cycling (Fig. 2). Overall, AOA abundance was positively correlated with all N cycling indicators (r between 0.61 and 0.58, Fig. 2a), and to a lesser extent soil organic C and N content. This correlation remains when only comparing savanna and *Eucalyptus* monocultures (Fig. S3). Bacteria abundance was negatively correlated with soil nitrate and inorganic N content, but also with nematode cp1 bacterial-feeders ($r = -0.40$), while fungi abundance did not correlate significantly with none of the variables measured. Cp1 bacterial-feeders correlated positively with both soil nitrate and inorganic N content, while cp2–4 bacterial-feeder abundance increased with soil organic C and N. On the other hand, the abundances of nematode fungal-feeders and omnivores and predators were negatively correlated with soil C, N, nitrate and inorganic N contents, but also with nitrification and net N mineralization rates (Fig. 2a). When only plantations were considered (i.e. excluding savanna dataset), the remaining correlations were the positive correlation between AOA abundance and soil nitrate, inorganic N content, nitrification and N mineralization rates; the positive correlation between cp1 bacterial-feeders and soil nitrate and inorganic N content and the

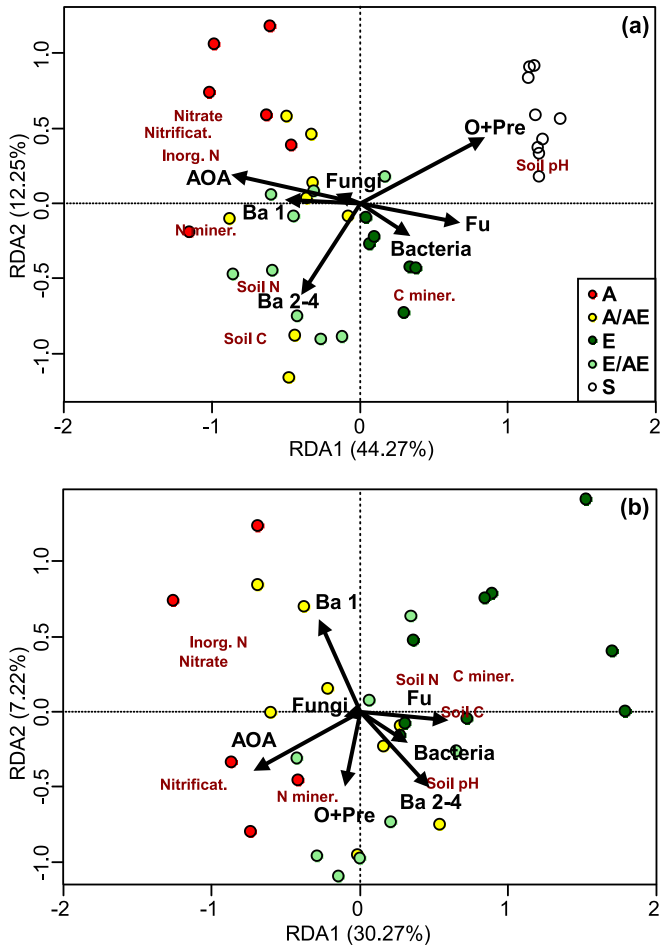

**Fig. 1.** Redundancy analysis of soil properties constrained with soil microbial and nematode community indicators for all land uses (a), and for plantations only (b). E: Eucalyptus monoculture; *E*-AE: under eucalyptus trees in mixed plantation; A-AE, under acacia trees in mixed plantation; A: acacia monoculture; S: herbaceous savanna. AOA: number of AmoA gene; Bacteria: number of 16S gene copies; Fungi: number of 18S gene copies; Ba1: cp1 bacterial-feeders nematodes; Ba2–4: cp2–4 bacterial-feeders nematodes, Fu: Fungal-feeders nematodes; O + Pr: Omnivores and Predatory nematodes.

negative correlation with bacteria abundance; and the positive correlation between cp2–4 bacterial-feeder abundance and soil C content (Fig. 2b).

## 4. Discussion

### 4.1. Savanna afforestation with Eucalyptus impacts on soil properties and micro-food web

In agreement with our first hypothesis, soil C and N contents were higher under plantations than under the native savanna, aligning with global findings on the impacts of afforestation on soil organic matter (Li et al., 2012; Paul et al., 2002). These changes have been attributed to higher C inputs under forested systems, a strong determinant of soil C storage (e.g. Fujisaki et al., 2018). We suspect that the change in quantity and quality of the organic matter restituted between savanna and plantations, combined by the fertilization of the plantations, have been large enough to impact on soil organic matter stock after >25 years of afforestation. Consistent with our study, d'Annunzio et al. (2008) compared native savannas and *Eucalyptus* plantations in Congo and reported C contents in the top 10 cm soil layer of 10.9 g C kg$^{-1}$ soil in 23-year-old eucalyptus unburnt plantations versus 5.5 g C kg$^{-1}$ soil in nearby savannas. Savannas from the study site experience periodic fires, which has significant negative impacts on plant litter restitution and soil organic matter (Jiang et al., 2022; Abbadie et al., 1992), while the level of litter restituted by plantations become substantial within a few years after their establishment (Nouvellon et al., 2012). Hence, Epron et al. (2009) have highlighted that the restitution of *Eucalyptus* litter is high enough to offset soil C losses from former savanna land use, even during the first plantation rotation. Consistent with previous observations by Li et al. (2012), soil C content have increased faster than soil N content with afforestation, resulting in a rise in the soil C:N ratios under plantations. Nonetheless, afforestation improved net N mineralization and nitrification rates and soil N availability. We expect this increase to be a combination of factors, such as the reduction of N loss through the

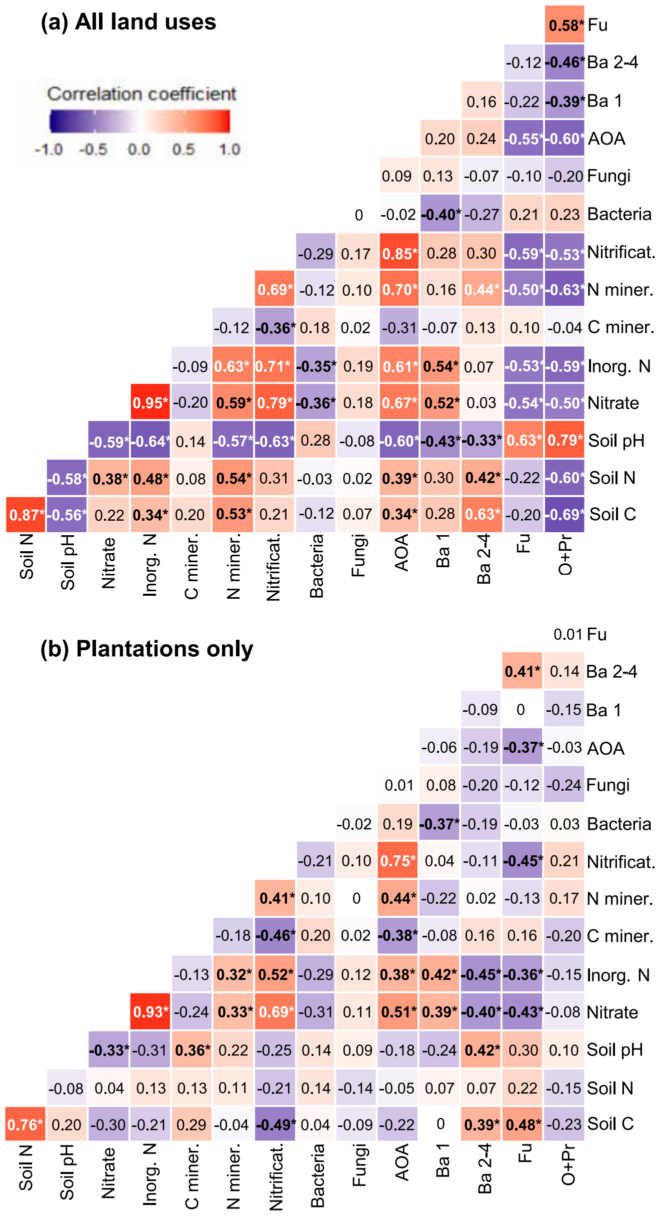

**Fig. 2.** Pearson correlation coefficient matrices of soil, microbial and nematode communities' properties for all land uses (a), and for plantations only (b). Correlations were corrected for multiple comparisons with the Benjamini-Hochberg method. Significant correlations ($p$-value <0.05) are written in boldface and bears an asterisk. AOA: number of AmoA gene copies; Bacteria: number of 16S gene copies; Fungi: number of 18S gene copies; Ba1: cp1 bacterial-feeders nematodes; Ba2–4: cp2–4 bacterial-feeders nematodes, Fu: Fungal-feeders nematodes; O + Pr: Omnivores and Predatory nematodes.

periodic fires (Jiang et al., 2022), along with the end of perennial grass inhibition of archaeal nitrification (Kaur-Bhambra et al., 2022; Lata et al., 2022; Srikanthasamy et al., 2018), as suggested by our observation of the increase in both AOA abundance and relative contribution of nitrification to total net N mineralization under plantations. Furthermore, the increased root exploration potential per trees allowed to sample N from deeper soil layers, that is then restituted to the superficial soil layer through litter restitution (Kellman, 1979). Finally, the increase of bacterial: fungal energy pathway ratio with savanna afforestation suggests a shift in N acquisition strategy from highly conservative under savanna (e.g. Lata et al., 2022) towards more acquisitive (de Vries and Bardgett, 2012).

Increase in AOA abundance with afforestation is indeed positively correlated with all the indicators related to N cycling – especially nitrification – in accordance with the knowledge of the key role of AOA in N cycling in these type of soils (Rughöft et al., 2016; Levy-Booth et al., 2014; Rachid et al., 2013) through nitrification. In line with our hypothesis, positive correlation of AOA with soil N availability (nitrate and total inorganic N content) was complemented by a positive influence of nematode cp1 bacterial-feeders. Positive association were often found between bacterial-feeders and N availability (Brondani et al., 2022; Ranoarisoa et al., 2018; de Vries et al., 2013), as bacteria grazing is known to stimulate bacterial activities and turn-over and increasing N release from bacterial necromass (Trap et al., 2016; Blanc et al., 2006; Ferris et al., 1998). In the case of our study, this role appears to be carried out by cp1 bacterial-feeders, which are supposedly more efficient to recycle microbial N thanks to their higher predation rates (e.g. Ferris et al., 2001), as suggested by the negative correlation between bacteria and cp1 bacterial-feeders abundance, while this relationship was not significant with cp2–4 bacterial-feeders. Overall, savanna afforestation benefits to the soil micro-food web likely had a positive feedback on N cycling, which is supported by studies in agroforestry using either correlative approach (e.g. Sauvadet et al., 2019) or nematode inoculation experimental approach (e.g. Trap et al., 2021).

### 4.2. *Acacia introduction impacts on N cycling linked with soil micro-food web changes*

In agreement with our second hypothesis, the presence of *Acacia*, either in mixture or in monoculture, led to higher net N mineralization and nitrification rates, and N availability in the topsoil compared to *Eucalyptus* monoculture. These results are in line with the extensive literature on the benefits of mixing eucalyptus with legume trees in N-deficient soils, aimed at partially meeting the strong N demand of fast-growing *Eucalyptus* trees (Cardoso et al., 2020). These benefits are due to both biological $N_2$ fixation of *Acacia*, estimated at 115 kg ha$^{-1}$ year$^{-1}$ in pure *A. mangium* plantation of 7 year old in Congo - Pointe Noire by Bernhard-Reversat (1996), and subsequent restitutions of N-enriched litter (Santos et al., 2017; Tchichelle et al., 2017). However, despite the increase in N cycling and N inputs with *Acacia* presence in *Eucalyptus* plantations, there were no significant changes in soil organic C or N contents in the topsoil between the plantation types after 7 years of plantation, similarly to Voigtlaender et al. (2019). This suggests a rapid turnover of the exceeding N returned under *Acacia* trees. The increase of net N mineralization and nitrification, in parallel with a decrease of net C mineralization from *Eucalyptus* to *Acacia* monocultures, implies that soil micro-food web composition may also play a crucial role to explain the shifts observed. Similar to the trends observed for savanna afforestation, *Acacia* introduction led to an increase in both AOA and cp1 bacterial-feeders abundance, which is commonly observed for AOA (Santana et al., 2021; Rachid et al., 2013). We expect that the higher quality litter restituted by *Acacia* compared to *Eucalyptus* trees favored the development of cp1 bacterial-feeders. The main drivers of N cycling within plantations soil micro-food web were also AOA for net N mineralization and nitrification rates, complemented by the positive influence of cp1 bacterial-feeders on nitrate and inorganic N availability, underlining the importance of both micro-food web components in this cycle (Lehtovirta-Morley, 2018; Trap et al., 2016).

We thus observed similar relationships between soil micro-food web components and N cycling indicators both for savanna afforestation with *Eucalyptus* and *Acacia* introduction within plantations, which partially contradicts our second hypothesis. We expected that N inputs increase with *Acacia* $N_2$ fixation would benefit to AOA, which in turn would exert positive feedbacks on N dynamics under these plantations. Nonetheless, we also assumed that fungal: bacterial energy channel ratio would decrease with the presence of *Acacia* in the plantations. We expect that (i) the fact that nitrification was carried in great majority by AOA and not AOB in these soils decreased the importance of bacteria in driving N cycling (at least regarding nitrification process) and (ii) that the relatively young age of the plantations with *Acacia* (7 years-old) did not allow soil micro-food web to fully stabilize. Indeed, amongst the nematode community, only bacterial-feeders benefitted from afforestation in our study; we speculate that omnivores, predators and fungal-feeders, of lower resilience than bacterial-feeders (Puissant et al., 2021; Postma-Blaauw et al., 2010) may develop and complexify soil micro-food web functioning at a later stage. Supporting this last assumption, we could not see significant changes in soil C content between plantations, which is usually found in young plantations (Mayer et al., 2020). Nevertheless, we may conclude that mixing *Acacia* with *Eucalyptus* improved N functions through biological N fixation, but also positive feedbacks from micro-food web differentiation, supporting the common findings that mixed plantations are more beneficial for soil fertility than monocultures (Rachid, 2020; Voigtlaender et al., 2019; Tchichelle et al., 2017).

## 5. Conclusion

This study highlights the interest to characterize soil micro-food web components in parallel to soil C and N cycling for different savanna afforestation modalities. The increase of soil organic C and N content, nitrification, N mineralization rates and N availability, with savanna afforestation – even with *Eucalyptus* monoculture – could be linked with different soil food web components and processes. Nitrification and secondarily N mineralization rates were effectively associated with AOA abundance increase, corresponding to the end of inhibition of this process by savanna grasses, but also the end of the disturbance caused by periodic fires. Yet, nematode bacterial-feeders were also strongly correlated with N availability increase, suggesting important feedbacks of microbial grazing in the change of N dynamics with afforestation. Similar soil food web – soil N cycling relationships could be observed when only focusing on *Acacia* introduction impacts on plantation functioning, suggesting that increased N inputs through $N_2$-fixation led to positive feedback from both the development of AOA and nematode microbial grazing. The importance of the role of nematodes in N-cycling is a strong incentive to consider them in plantation management, for instance by avoiding soil acidification or providing soil cover to preserve this community.

## CRediT authorship contribution statement

**M. Sauvadet:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **J.M. Harmand:** Writing – review & editing, Methodology, Investigation, Conceptualization. **P. Deleporte:** Writing – review & editing, Visualization, Investigation, Data curation. **A. Martin:** Formal analysis. **F. Zarah-Shailia:** Formal analysis. **C. Villenave:** Writing – review & editing, Visualization, Formal analysis, Data curation. **A. Jimenez:** Visualization, Formal analysis, Data curation. **L. Mareschal:** Writing – review & editing, Methodology, Investigation, Conceptualization. **J.P. Bouillet:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization. **J.P. Laclau:** Writing – review & editing, Methodology, Investigation, Conceptualization. **C. Plassard:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Conceptualization. **J. Trap:** Writing – review & editing, Investigation. **A. Robin:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Data curation, 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.

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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.2025.106027.

## Data availability

Data will be made available on request.

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