N-microbial properties are key indicators of fertility in maturating soils built for urban greening.

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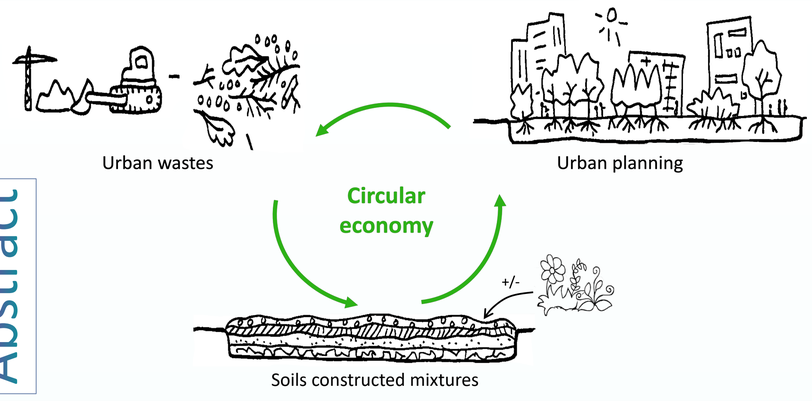
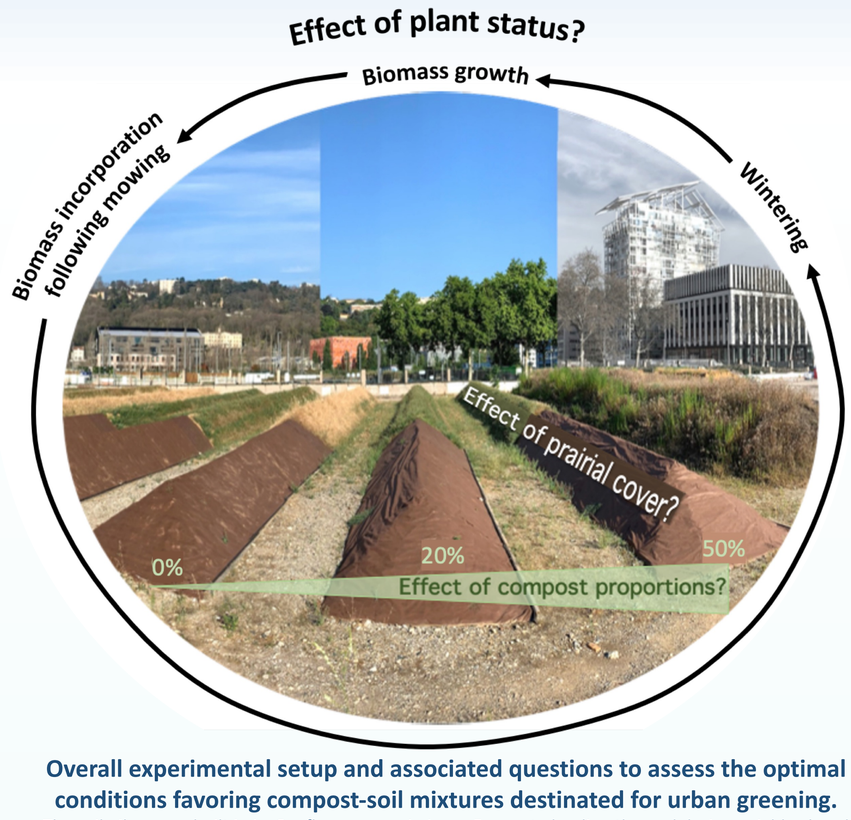


Abstract

Soils constructed from urban planning wastes provide a sustainable solution for urban planners to design urban green spaces that meet the growing demand for nature in the city while limiting artificialization of peri-urban agricultural areas. However, while the pedological and chemical characteristics of these constructed soils are easily assessed, their biological fertility, and especially their nitrogen (N) availability, remains essentially associated to their inhabiting microbial communities. Accordingly, the stability of microbial N-functioning and the prerequisite time to provide sufficient N to plant covers is an under-explored ecological question.

To address this issue, nine soils were constructed from compost, topsoil, and deep soil mixed in different proportions. Half were hydroseeded once with a mixture of annual and perennial plants. We then monitored soil fertility for 24 months, to cover two growing and two wintering seasons, and assess N cycle seasonality in regards of plant community development and pedological and chemical characteristics. Free-living microbial N-fixation, nitrification and denitrification enzyme activities were measured in soil incubations following acetylene reduction assays, colorimetric quantifications of nitrite/nitrate accumulation, and N_2O gas chromatography, respectively. Relative abundances of genes associated to these enzyme activities were assessed using targeted quantitative PCR.

Our results suggest microbial indicators of soil functioning are truly relevant to monitor biological fertility of constructed soils in urban ecosystems.

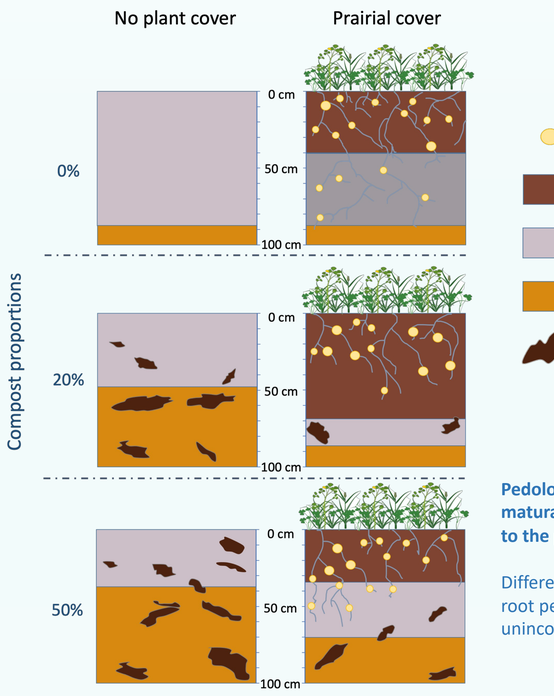


Overall experimental setup and associated questions to assess the optimal conditions favoring compost-soil mixtures destinated for urban greening.

The soil piles were built in La Confluence area in Lyon, France to be directly used during neighborhood rehabilitation. They were kept for maturation and scientific monitoring from November 2019 to March 2022 (24 months).

I - Fertility characterization by physical and chemical properties

Physical properties

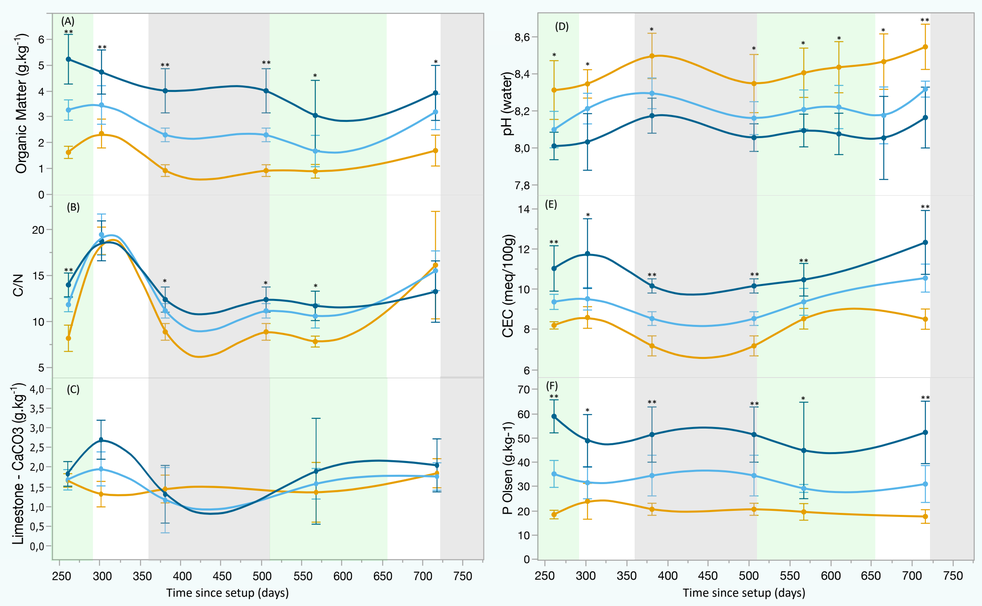


Pedological observations after 18 months maturation of constructed soil profiles according to the proportion of compost and prairial cover

Different horizons were identified according to root penetration, settling, and wet spots, as well as unincorporated clod-forming compost.

Prairial cover influenced compost incorporation, porosity, and moisture loss.

Chemical properties

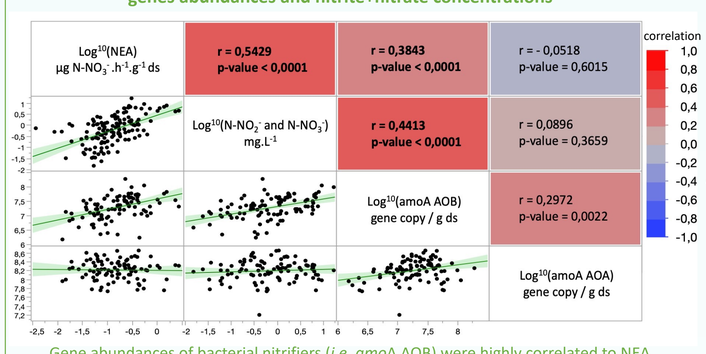


Agronomic monitoring of soil fertility during 24 months based on conventional chemical indicators

Organic matter content (A), C/N ratio (B), Limestone concentration (C), pH measured in water (D) Cation Exchange Capacity (CEC) (E) and P_2O_5 concentration (F).
All indicators are presented according to proportions of compost and considering plant growth season (wintering: grey; biomass growth: green; biomass incorporation following mowing: white).

Despite seasonal fluctuations, all chemical agronomic indicators remained stable during 24 months. Higher proportions of compost implied higher organic matter content, total N and P_2O_5 concentrations and CEC. pH was lower in soils harboring higher proportions of compost.

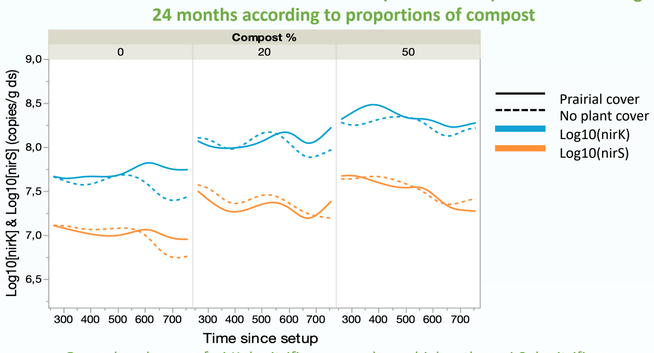
Spearman correlations between nitrification enzymatic activity (NEA), genes abundances and nitrite+nitrate concentrations



Gene abundances of bacterial nitrifiers (*i.e. amo*A AOB) were highly correlated to NEA and nitrite + nitrate concentrations. Gene abundances of archaeal nitrifiers (*i.e. amo*A AOA) were only correlated to AOB gene abundances.

AOB gene abundances mostly explained NEA

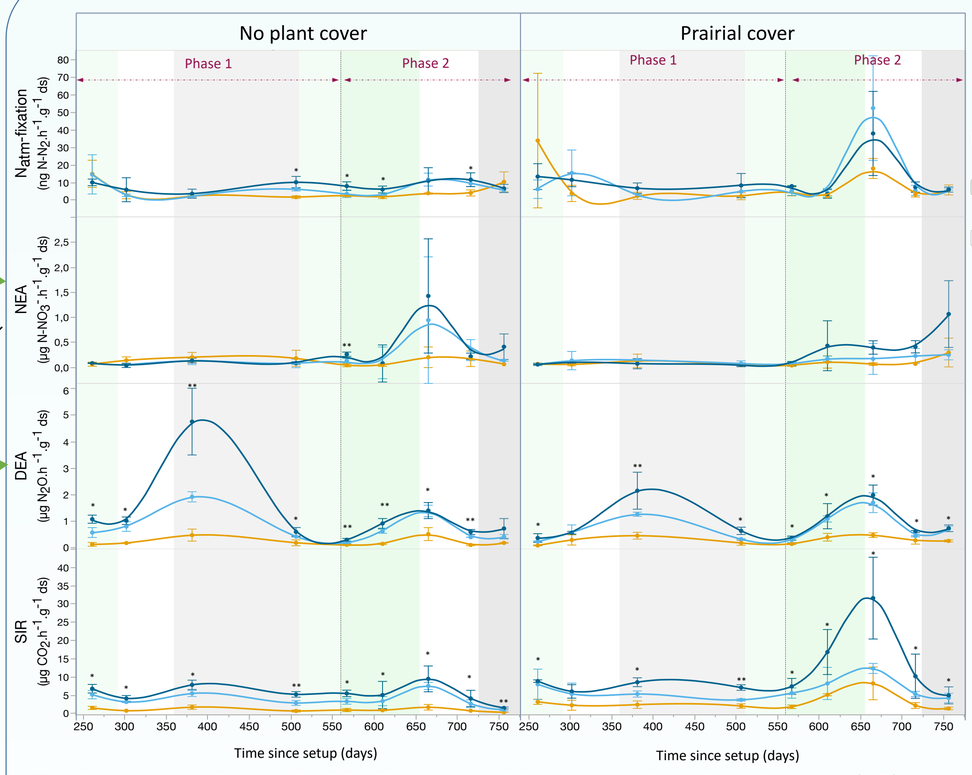
Gene abundances of bacterial denitrifiers (*nir*K and *nir*S) monitored during 24 months according to proportions of compost



Gene abundances of *nir*K denitrifiers were always higher than *nir*S denitrifiers. All gene abundances increased with proportion of compost

Higher *nir*K gene abundances with higher proportions of compost

II – Biological fertility characterization by microbial activities



Microbial-related indicators of soil fertility monitored during 24 months with no plant cover (left) vs. prairial cover (right)
Rate of N_{atm}-fixation activity (A), Nitrification Enzyme Activity (**NEA**, B), Denitrification Enzyme Activity (**DEA**, C) and Substrate Induced Respiration (**SIR**, D). All indicators are presented according to proportions of compost, considering plant growth season (wintering: grey; biomass growth: green; biomass incorporation following mowing: white) and split in two maturation phases.

Two maturation phases

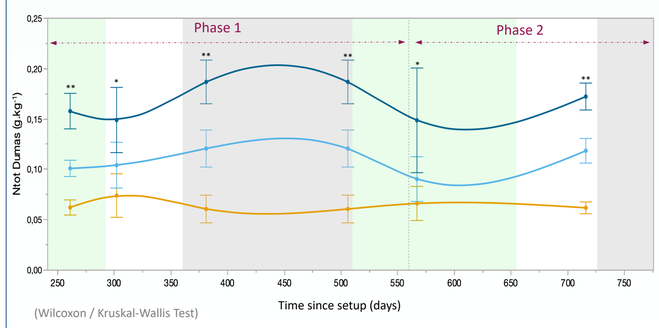
Rates of NEA, N_{atm}-fixation and SIR enzymatic activities remained low during the first 18-month maturation (phase 1), and drastically increased after (phase 2), especially under prairial cover for N_{atm}-fixation (at 665 days) and SIR (from 610 to 756 days).

Prairial cover decreased rate of **DEA (phase 1)**

Suggesting a competition between plants and denitrifiers for the nitrate resource

Addition of compost increased heterotrophic activities (i.e. DEA and SIR)

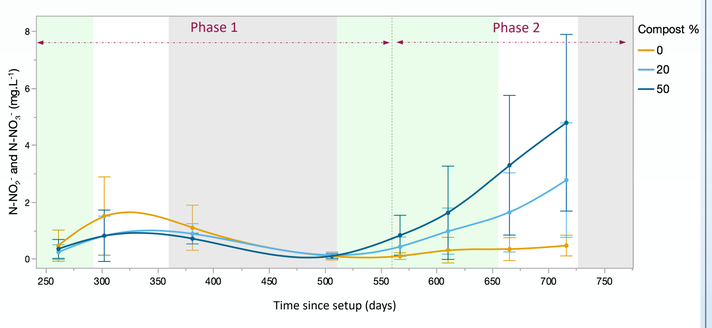
Total N concentrations according to proportions of compost monitored during 24 months



(Wilcoxon / Kruskal-Wallis Test)

Despite slight seasonal fluctuations, N_{tot} concentrations remained non significantly different according to proportions of compost with higher N_{tot} in higher proportion of compost.

Mineral N substates (nitrite and nitrate) concentrations according to proportions of compost monitored during 24 months



Nitrite and nitrate concentrations increased significantly in soil mixtures including compost after 18 months maturation (phase 2).

While N_{tot} concentration showed the effect of the proportion of compost, nitrite and nitrate concentrations, associated with microbial communities, better revealed N-availability for plants

Conclusion

Agronomic indicators, usually monitored for soil fertility, showed **no significant difference** during 24-months maturation, but some of them underlined the effects of proportions of compost and prairial cover.

Microbial properties more precisely showed the soil maturation phases:

- Initial materials effect : with heterotrophic microbial activities (DEA, SIR and to a lower degree N_{atm}-fixation)
- Plant cover benefits : on all microbial activities at different levels
- Soil N-valability better disclosed by NEA
- AOB –nitrifiers gene abundance could reveal variations in NEA
- Best time to setup : when N-enrichment activities were higher