SCAP
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Land use change effects on soil physical-chemical parameters and microbial communities on a tropical ecosystem from Guinea-Bissau

Efeitos da alteração do uso do solo nos parâmetros físico-químicos e na comunidade microbiana do solo num ecossistema tropical da Guiné-Bissau

Rafael António[1], Ana João Martins[1], Inês Cordeiro[1], Eduardo Onofre Feijão[2], Ana Rita Matos[3], Filipa Monteiro[4,5] & Mónica Sebastiana[3,*]

[1] *Faculdade de Ciências da Universidade de Lisboa, 1749-016 Lisboa, Portugal*
[2] *Marine and Environmental Sciences Centre (MARE), Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal*
[3] *Instituto de Biosistemas e Ciências Integrativas (BioISI), Departamento de Biologia Vegetal, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal*
[4] *Linking Landscape, Environment, Agriculture and Food (LEAF), Associate Laboratory TERRA, Instituto Superior de Agronomia (ISA), Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal*
[5] *Centre for Ecology, Evolution and Environmental Changes (cE3c) & Global Change and Sustainability Institute (CHANGE), Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal*
*(*Corresponding author: mgsebastiana@fc.ul.pt)*
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ABSTRACT

Current studies show a decline in soil biodiversity, which puts at risk future food production since soil organisms play a key role in essential ecosystem functions such as, nutrient cycling and soil fertility, soil carbon sequestration, climate regulation, litter decomposition, erosion, water retention, interaction with plants, among others. This study aims to evaluate how the conversion of a tropical native forest into a field for agricultural production impacts the soil physical-chemical parameters and how these correlates with the abundance of specific group of microorganisms. The soils analyzed were collected in Guinea-Bissau, at 3 locations with different land uses: a primary forest, an annual crop field (peanut) and a perennial crop field (cashew), during the wet and dry season. We analyzed several soil parameters including pH, water content, N, P and C contents, soil respiration, and soil protein index. Mycorrhizal spore density, analysis of soil PLFA (Phospholipid fatty acids), and qPCR targeting genes involved in N, C and P cycling, were selected for investigating changes in soil microbial diversity. Our results confirm that land use changes alter soil physically and chemically, and its microbial community as well.

Keywords: soil biodiversity, land use, PLFA, tropical soils, West Africa

RESUMO

Estudos recentes mostram um declínio na biodiversidade do solo, o que coloca em risco a produção futura de alimentos, uma vez que os organismos do solo são responsáveis por funções essenciais, como os ciclos de nutrientes e a fertilidade do solo. Este estudo visa avaliar como é que a conversão de uma floresta tropical nativa num campo agrícola, altera os parâmetros físico-químicos do solo e como é que estes se correlacionam com a abundância de grupos de microorganismos específicos. Os solos analisados foram recolhidos na Guiné-Bissau em 3 locais com diferentes usos do solo: floresta primária, cultura anual (amendoim) e cultura perene (caju), durante as épocas húmida e seca. A análise dos parâmetros do solo inclui a determinação do pH, respiração do solo, conteúdo hídrico, índice proteico, e conteúdo em nutrientes (P, N e C). Para avaliar as alterações na diversidade microbiana do solo foram selecionadas análises de PLFA (*Phospholipid fatty acids*), PCR quantitativo de genes envolvidos nos ciclos de nutrientes (N, P e C) e determinação da densidade de esporos de fungos micorrízicos. Os resultados obtidos confirmam que a conversão de floresta para a agricultura altera os parâmetros do solo e as suas comunidades microbianas.

Palavras-chave: biodiversidade do solo, uso do solo, PLFA, solos tropicais, África Ocidental.

INTRODUCTION

The anthropogenic intervention on native ecosystems is occurring worldwide at an alarming rate (Wood *et al*., 2017). Due to the increasing demand for resources like food, fibers and fuels, sensitive and complex ecosystems like forests, mangroves and grasslands are being converted into croplands and pastures (Dinesh *et al*., 2004). Between 1980 and 2000, on tropical Africa, 55% of the new agriculture land was derived from pristine forests and 28% from disturbed forests (Gibbs *et al*., 2010).

Despite the benefit for the people, especially in poor and developing countries, this land use conversions lead to a decrease in soil quality and soil biodiversity (Dinesh *et al*., 2004). This negative throwback is especially important not only because their decline puts at risk the future food production (van Gestel *et al*., 2021) but also because soil microorganisms play key roles in essential ecosystem functions such as nutrient cycling and soil fertility, soil carbon sequestration, climate regulation, erosion, water retention, interaction with plants, among others (Bach *et al*., 2020).

The present study aims to understand how the change of land use in a tropical ecosystem from Guinea-Bissau affects the physical-chemical parameters of soil and its microbial communities.

MATERIALS AND METHODS

Study area, soil sampling and treatment

Soil samples were collected from three different locations with different land uses: a primary forest, a perennial crop field (cashew) and an annual crop field (peanut). Samples were collected at 0-20 cm depth during the dry season (November 2021) and the wet season (October 2022). Five soil replicates, each consisting of a pool of five soil samples, were collected at each location. Samples for biological analysis, except for arbuscular mycorrhizal fungi (AMF) spore abundance determination, were frozen and stored at -20ºC. All the other samples were weighted and left undisturbed to completely air dry. After this period, samples were sieved through a 2mm sieve and stored at 4ºC.

Soil chemical analysis

Soil active carbon content was determined using air dried soil, by the quantification of potassium permanganate oxidation with a spectrophotometer (Weil *et al*., 2003). The soil moisture content was determined by the gravimetric water content procedure. pH was determined using an electrode pH meter and soil suspended in deionized water (1:1), after shaking for 30 minutes. N concentration was estimated following the Kjeldahl method (Bremner & Mulvaney, 1982). $N\text{-}NO_3^-$ was quantified according to Singh (1988). Available P was determined by the Egner-Riehm Double-Lactate DL method (Egnér *et al*., 1960). Soil organic carbon was analyzed by the Dumas dry combustion method (FAO, 2019).

Soil Biological Analysis

Soil protein content was determined by the quantification of the autoclaved-citrate extractable (ACE) soil protein index using the Bradford spectrophotometric assay at 595 nm for protein quantification, according to a procedure adapted from Wright and Upadhyaya (1996).

Soil respiration was determined by capturing and quantifying the CO_2 produced after re-wetting dried soil samples, using the sealed chamber alkali trap respirometry method adapted from Zibilske *et al*. (1994).

AMF spore abundance was determined following the wet sieving (45–700 μm mesh size) and decanting method followed by sucrose density centrifugation (INVAM), using 100 g of dried soil. The spores were counted under a stereomicroscope.

Soil samples were freeze-dried and used for PLFA assays according to established methods (Quideau *et al*., 2016). Lipids were extracted from 4 g of freeze-dried soil and fractionated using SPE columns. After methylation, phospholipids were separated using an HP GC-FID gas chromatography (Agilent Technologies Inc., Santa Clara, CA, USA). Phosphatidylcholine (PC19:0) was used as an internal standard. Soil total microbial biomass was calculated using the sum of all detected PLFAs in a sample. PLFAs were quantified using

the areas under the different GC chromatogram peaks (% response).

DNA was extracted from 0.15 g of -20 stored soil using a commercial kit (DNeasy PowerSoil Pro Kit). DNA concentration was evaluated using a nanodrop spectrophotometer. DNA integrity was evaluated by agarose gel electrophoresis.

Statistical analysis

In order to access normality and homogeneity of variance of the data, Shapiro-Wilk and a Levene's tests were performed, respectively. To evaluate the differences between soil uses a One-Way ANOVA or a Kruskal-Wallis U test was conducted followed by a Tukey HSD test or a Mann-Whitney--Wilcoxon test, respectively. Statistical significance was considered for $p<0.05$.

RESULTS

Land use change from native forest to crop field resulted in significant changes in the soil chemical and biological properties (Table 1 and 2). In general, our data shows that forest soil has significant higher values ($p < 0,05$) for most of the parameters analyzed such as protein, active carbon, organic matter, water content and respiration values, compared to peanut field and cashew field. On the other hand, soil from cashew field has less P_2O_5 than forest ($p = 0.012$) and has significant lower values ($p < 0,05$) in parameters such as protein, active carbon, AMF spore abundance and water content. Finally, the peanut field soil has significant higher contents ($p < 0,05$) of N- NO_3^- and AMF spore abundance. No significant differences between the different soil uses were found regarding the soil DNA concentration, pH, total N and N-NH_4^+ content.

PLFA preliminary analysis (Table 3 and 4) shows a higher total microbial biomass for forest soil samples, independently of season. Comparison between seasons shows that there is not much change in microbial biomass from one season to the other. In the wet season the peanut field had higher levels of microbial biomass compared to the cashew field, whereas in the dry season the peanut field had less microbial biomass than the cashew field. The recognized biomarker for saprophytic

Table 1 - Soil chemical analysis along with statistical information. Values correspond to the means ± SD

Soil use	Relative Humidity	pH	Active Carbon	P_2O_5	N Kjeldahl	N-NH_4	N-NO_3
	%		mg/Kg	mg/Kg	mg/Kg	mg/Kg	mg/Kg
Forest	13.38 ± 1.78	5.29 ± 0.09	249.52 ± 19.28	9.1 ± 1.095	270.2 ± 51.05	5.52 ± 1.58	1.01 ± 0.63
Cashew	9.73 ± 1.01	4.90 ± 0.17	61.28 ± 12.56	6.91 ± 0.57	217.34 ± 56.36	6.83 ± 2.24	1.20 ± 0.58
Peanut	11.98 ± 0.78	5.15 ± 0.32	159.89 ± 25.97	7.46 ± 0.78	261.84 ± 65.02	4.05 ± 1.12	4.98 ± 0.38
Stat Test	ANOVA	ANOVA	ANOVA	ANOVA	ANOVA	ANOVA	ANOVA
p-value	< 0,001	0,075	< 0,001	0,014	0,468	0,111	< 0,001

Table 2 - Soil biological analysis along with statistical information. Values correspond to the means ± SD

Soil use	DNA concentration	AMF spore abundance	Organic Matter (C%)	Respiration	Protein
	ng DNA/g soil	nº spores/g soil	%	mg CO_2/g soil	mg protein/g soil
Forest	4975 ± 3021,75	23.49 ± 0.19	0.86 ± 0.04	0.97 ± 0.22	2.57 ± 0.23
Cashew	4527 ± 1789,04	14.36 ± 1.81	0.65 ± 0.08	0.66 ± 0.10	1.63 ± 0.23
Peanut	4503 ± 1682,11	63.09 ± 9.94	0.72 ± 0.06	0.53 ± 0.07	1.99 ± 0.1
Stat Test	ANOVA	Kruskal-Wallis	ANOVA	Kruskal-Wallis	ANOVA
p-value	0,933	0,002	< 0,001	0,008	< 0,001

fungi, C18:1ω9c, was more abundant in the forest, independently of the season. C18:2ω6c, which is used to estimate the biomass of saprophytic and ectomycorrhizal fungi was higher in the cashew field during the wet season, whereas in the dry season the peanut field had the highest values. The highly abundant bacterial and fungal biomarker (C16:0) does not seems to be affected by land use. A biomarker for protist (C20:3) was detected, showing higher levels in peanut and cashew fields, especially during the wet season.

Table 3 - Soil biomass during the wet and dry season based on PLFA analysis. Only one sample was analyzed from each soil type and season

Soil Use	Soil Biomass μg Fatty Acids/g soil	
	Wet Season	Dry Season
Forest	11,144	12,816
Cashew	9,709	11,993
Peanut	10,938	9,013

use conversion leads to changes soil physico-chemical properties and in soil microbial communities.

As previously demonstrated by other authors, our results showed a higher content of protein in forest soils compared to soils from cashew or peanut fields (Williams *et al*., 2020). This elevated content of protein may be derived from an abundant and diverse community of microorganisms that is common in these natural undisturbed soils (Williams *et al*., 2020). In agreement, the high levels of soil respiration detected in our study in forest soils indicates a higher microbial activity and a more abundant community of microorganisms in these soils (Williams *et al*., 2020).

Surprisingly, we didn't find significant differences in soil DNA concentration between soils from forest, peanut field, and cashew field. This could be related to a limited ability of DNA purification kits to extract the total amount of DNA from our soil samples.

Table 4 - Some soil fatty acids identified in soil during the wet and dry season based on PLFA analysis. Only one sample was analyzed from each soil type and season

Soil Use	C15:0 %		C16:0 %		C17:0 %		C18:1ω9c %		C18:2ω6c %		C20:3 %	
	Wet Season	Dry Season	Wet Season	Dry Season	Wet Season	Dry Season	Wet Season	Dry Season	Wet Season	Dry Season	Wet Season	Dry Season
Forest	0,8	0,85	21,5	21,09	1,7	1,78	2,9	2,27	5,6	2,79	4,9	3,72
Cashew	1,0	0,92	20,5	22,32	1,3	1,59	1,7	1,14	7,7	2,53	7,2	5,41
Peanut	0,9	1,00	20,5	20,73	1,5	1,27	1,9	0,80	5,4	5,72	5,5	5,53

DISCUSSION

The current literature shows that conversion of native ecosystems like forests into croplands is deeply associated with a decrease in soil biodiversity and soil quality (Dinesh *et al*., 2004). This negative impact jeopardizes the soil viability for future generations and puts at risk the future food production (van Gestel *et al*., 2021). To study how the conversion of a forest into two different crop fields affects soil quality and biodiversity we selected and analyzed several physico-chemical and biological parameters. Like other studies (Chen *et al*., 2001; Dinesh *et al*., 2004; Williams *et al*., 2020), our findings revealed that land

Some authors reported higher values of AMF spore abundance in highly disturbed soils rather than in native and biodiverse environments, such as forests (Ontivero *et al*., 2022). Land use change, native vegetation replacement and soil disturbance are identified as factors that favor the sporulation of certain species (Ontivero *et al*., 2022). Our results follow this trend since the more disturbed peanut soils (monoculture) presented the higher values. On the other hand, spore richness could be related to the fact that peanut is a leguminous plant that forms abundant mycorrhizas with AM fungi. Cashew fields presented the lowest values of AMF spore abundance, which might indicate a

lower ability of cashew trees to be colonized by AMF, compared to peanut, an herbaceous plant.

The labile pool of soil carbon is known to be affected by land use changes and agriculture management practices, being used as a soil quality indicator (Geraei *et al*., 2016). As demonstrated by other authors, our findings also show higher values of organic and active carbon in the undisturbed forest soils. Peanut and cashew field other soils had lower contents of carbon which is probably related to the lower biomass addition to soil since crop residues are usually removed (Geraei *et al*., 2016).

The different nitrogen pools are also deeply related and influenced by land use changes and the soil microbial community, and so can be used as soil quality and fertility indicators (Yang *et al*., 2010). These different nitrogen forms are greatly associated with the activities of the microbial communities that control N mineralization, nitrification, and the uptake of inorganic N by microbes or plants (Yang *et al*., 2010). Although other studies showed greater values of total N in undisturbed soils and native ecosystems, and greater values of $N\text{-}NH_4^+$ in croplands, related to chemical fertilization (Yang *et al*., 2010), our results didn't reveal any significant differences between soil uses. On the other hand, our $N\text{-}NO_3^-$ results are in accordance with the literature, being higher in the peanut and cashew field soils than in undisturbed forest soils. These results might indicate that nitrogen was transformed mainly in the form of nitrate, most probably related with a greater community of microorganisms that regulate this conversion (nitrification) (Yang *et al*., 2010). Studies have shown that following clear cutting of forests there is an increase in soil NO_3^- related to increased nitrification rates and higher abundance of nitrifying bacteria (Qi *et al*., 2021).

CONCLUSION

The conversion of forests into crop fields is often associated with a decline in soil biodiversity, soil quality and fertility. Our study on a tropical ecosystem from Guiné-Bissau in Africa shows that forest soils have an overall higher values for most physico-chemical parameters, while peanut and cashew soils have lower values, with some exceptions, such as $N\text{-}NO_3^-$. This clearly indicates a decrease in soil quality following the conversion of forests to crop fields. Regarding soil biodiversity, our results point to a greater soil biodiversity in forest soils, and we expect that PLFA analysis will support and enhance this finding. Our preliminary results also support a decline in soil biodiversity following the conversion of forest to crop field.

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REFERENCES

Bach, E.M.; Ramirez, K.S.; Fraser, T.D. & Wall, D.H. (2020) - Soil biodiversity integrates solutions for a sustainable future. *Sustainability*, vol. 12, n. 7, p. 2662. https://doi.org/10.3390/su12072662

Bremner, J.M. & Mulvaney, C.S. (1982) - Nitrogen-Total. *In*: Page, A.L.; Miller, R.H. & Keeney, D.R. (Eds.) - *Methods of soil analysis. Part 2. Chemical and microbiological properties*. Soil Science Society of America, Madison, Wisconsin, p. 595-624.

Chen, G.; Gan, L.; Wang, S.; Wu, Y. & Wan, G. (2001) - A comparative study on the microbiological characteristics of soils under different land-use conditions from karst areas of southwest China. *Chinese Journal of Geochemistry*, vol. 20, n. 1, p. 52-58. https://doi.org/10.1007/BF03166849

Dinesh, R.; Ghoshal Chaudhuri, S. & Sheeja, T.E. (2004) - Soil biochemical and microbial indices in wet tropical forests: effects of deforestation and cultivation. *Journal of Plant Nutrition and Soil Science*, vol. 167, n. 1, p. 24-32. https://doi.org/10.1002/jpln.200321254

Egnér, H.; Riehm, H. & Domingo, W.R. (1960) - Untersuchungen uber die chemishe bodenanalyse als grundlage fur die beurteilung des nahrstoffzustandes der boden. II. Chemische extraktions-methoden zu phosphor – and kalimbestimmung. *Kungliga Lantbrukshögskolans Annaler*, vol. 26, p. 204-209.

FAO (2019) - *Standard operating procedure for soil total carbon*. Food and Agriculture Organization of the United Nations.

Geraei, D.S.; Hojati, S.; Landi, A. & Cano, A.F. (2016) - Total and labile forms of soil organic carbon as affected by land use change in southwestern Iran. *Geoderma Regional*, vol. 7, n. 1, p. 29-37. https://doi.org/10.1016/j.geodrs.2016.01.001

Gibbs, H.K.; Ruesch, A.S.; Achard, F.; Clayton, M.K.; Holmgren, P.; Ramankutty, N. & Foley, J.A. (2010) - Tropical forests were the primary sources of new agricultural land in the 1980s and 1990s. *Proceedings of the National Academy of Sciences of the USA*, vol. 107, n. 38, p. 16732-16737. https://doi.org/10.1073/pnas.0910275107

Ontivero, R.E.; Risio, L.V.; Iriarte, H.J. & Lugo, M.A. (2022) - Effect of Land-Use Change on Arbuscular Mycorrhizal Fungi Diversity in an Argentinean Endemic Native Forest. *Biology and Life Sciences Forum*, vol. 15, n. 1, art. 15. https://doi.org/10.3390/IECD2022-12430

Qi, D.; Feng, F; Fu, Y. & Sun, X. (2021) - Changes in soil ammonia oxidizers and potential nitrification after clear-cutting of boreal forests in China. *World Journal of Microbiology and Biotechnology*, vol. 37, art. 126. https://doi.org/10.1007/s11274-021-03087-2

Quideau, S.A.; McIntosh, A.C.; Norris, C.E.; Lloret, E.; Swallow, M.J. & Hannam, K. (2016) - Extraction and analysis of microbial phospholipid fatty acids in soils. *JoVE Journal of Visualized Experiments*, vol. 114, p. 54360. https://doi.org/10.3791/54360

Singh, J.P. (1988) - A rapid method for determination of nitrate in soil and plant extracts. *Plant and Soil*, vol. 110, n. 1, p. 137–139. https://doi.org/10.1007/BF02143549

van Gestel, C.A.; Mommer, L.; Montanarella, L.; Pieper, S.; Coulson, M.; Toschki, A.; Rutgers, M.; Focks, A & Römbke, J. (2021) - Soil biodiversity: State-of-the-art and possible implementation in chemical risk assessment. *Integrated Environmental Assessment and Management*, vol. 17, n. 3, p. 541-551. https://doi.org/10.1002/ieam.4371

Weil, R.R.; Islam, K.R.; Stine, M.A.; Gruver, J.B. & Samson-Liebig, S.E. (2003) - Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. *American Journal of Alternative Agriculture*, vol. 18, n. 1, p. 3-17. https://doi.org/10.1079/AJAA200228

Williams, H.; Colombi, T. & Keller, T. (2020) - The influence of soil management on soil health: An on-farm study in southern Sweden. *Geoderma*, vol. 360, art. 114010. https://doi.org/10.1016/j.geoderma.2019.114010

Wood, S.A.; Gilbert, J.A.; Leff, J.W.; Fierer, N.; D'Angelo, H.; Bateman, C.; Gedallovich, S.M.; Gillikin, C.M.; Gradoville, M.R.; Mansor, P.; Massmann, A.; Yang, N.; Turner, B.L.; Brearley, F.Q. & McGuire, K.L. (2017) - Consequences of tropical forest conversion to oil palm on soil bacterial community and network structure. *Soil Biology and Biochemistry*, vol. 112, p. 258-268. https://doi.org/10.1016/j.soilbio.2017.05.019

Wright, S.F. & Upadhyaya, A. (1996) - Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. *Soil Science*, vol. 161, n. 9, p. 575-586. https://doi.org/10.1097/00010694-199609000-00003

Yang, L.; Zhang, F.; Gao, Q.; Mao, R. & Liu, X. (2010) - Impact of land-use types on soil nitrogen net mineralization in the sandstorm and water source area of Beijing, China. *Catena*, vol. 82, n. 1, p. 15-22. https://doi.org/10.1016/j.catena.2010.04.004

Zibilske, L. (1994) - Carbon mineralization. *In:* Weaver, R.W.; Angle, S.; Bottomley, P.; Bezdicek, D.; Smith, S.; Tabatabai, A. & Wollum, A. (Eds.) - *Methods of soil analysis. Part 2. Chemical and microbiological properties*. Soil Science Society of America, Madison, Wisconsin, p. 835-863.