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Rezaei Rashti, Mehran, Nelson, Paul, Lan, Zhongming, Su, Ninghu, Esfandbod, Maryam, Liu, Xiangyu, Goloran, Johnvie, Zhang, Hanzhi, and Chen, Chengrong (2024) ***Sugarcane cultivation altered soil nitrogen cycling microbial processes and decreased nitrogen bioavailability in tropical Australia.*** **Journal of Soils and Sediments, 24 pp. 946-955.**

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https://doi.org/10.1007/s11368%2D023%2D03704%2D7

Sugarcane cultivation altered soil nitrogen cycling microbial processes and decreased nitrogen bioavailability in tropical Australia

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Abstract

Purpose: Land use conversion of natural ecosystems to intensive agriculture can alter soil biogeochemical processes and nutrient cycling, while increasing potential for land degradation. Disturbance of soil microbial community, due to land use conversion, may also lead to detrimental effects on soil ecosystem processes and services. Therefore, the objective of this study was to examine how sugarcane cultivation alters soil nitrogen (N) bioavailability and associated microbial processes in tropical Australia. Methods: Two adjacent paired sites (native forest vs sugarcane cultivation for 78 years; pasture vs sugarcane cultivation for 78 years) were selected and five composite surface soils (0-10 cm) were collected from each site. Results: Sugarcane cultivations decreased total organic carbon (OC; 45% - 48%), total N (51% - 54%), and total phosphorus (P; 26% - 37%) pools compared with native forest and pasture. Total mineral N (NH_4^+-N + NO_3^--N), dissolved organic C and N contents and

cumulative aerobic respiration were also lower in sugarcane than native forest and pasture lands. Reduction in soil microbial biomass (60%) following long-term conversion to sugarcane has resulted in higher metabolic quotient (qCO_2) and lower C use efficiency, indicating higher level of environmental stresses in sugarcane sites. Among N cycling associated genes, only *narG* showed significantly higher abundance in sugarcane than pasture land use, while *narG*, *nosZ*, *nirK* and *nirS* genes had significantly lower copy numbers in sugarcane compared with native forest. Conclusion: sugarcane cultivation decreased soil health and biochemical quality, N bioavailability, and N cycling processes in tropical climate of this study.

Keywords: Land use change; carbon and nitrogen bioavailability; functional gene abundance; sugarcane; pasture; native forest

1. Introduction

Changes in land use are among the most impactful human activities affecting ecosystem functions and biodiversity. Conversion of natural ecosystems, such as native forests, to pasture and cropping systems (e.g., sugarcane) not only results in significant alteration of soil structure, and carbon (C) and nutrient cycles, but also affects soil greenhouse gas emissions, and function of soil microbial communities (Don et al., 2011; Nacke et al., 2014; Tosi et al., 2016; Gava et al., 2022). Long-term monoculture based agricultural systems also generally enhance microbial decomposition of soil organic carbon (OC), and consequently reduce the sustainability of soil biogeochemical processes and nutrient cycling, while increasing the potential for soil erosion (Zatta et al., 2014; Qin et al., 2016). Therefore, better understanding of the long-term effects of land use change on soil physicochemical and biological properties plays a crucial role in optimum management of land resources for confronting issues related to future climate change and food security (Foley et al., 2011; Liu et al., 2018a).

Sugarcane is an important tropical crop (> 20 million ha), with its global production (> 2 billion tons) increased more than four times since 1965 (McKay et al., 2016) to meet the ever-growing demand for food and biofuel. Australia is the 7th biggest producer of sugarcane (after Brazil, India, China, Thailand, Pakistan, and Mexico; Linnenluecke et al., 2018), with around 4000 sugarcane farms (20 - 200 ha in size) operating along the tropical to subtropical regions of Australia's eastern seaboard. Most of Australian sugarcane was established on native forests or woodlands. In recent decades, these sugarcane lands received on average 100 - 250 kg nitrogen (N) ha^{-1} $crop^{-1}$ as synthetic N fertilisers (Thorburn et al., 2011; Armour et al., 2013), that could potentially affect their soil C and N stocks and turnover. Sugarcane cropping provides significant economic benefits for Australia; however, the potential environmental issues associated with this monoculture farming system, such as decline of soil fertility due to intensive management (e.g., fertilisation, irrigation, cultivation, removal of harvested biomass), increased greenhouse gas emissions, and runoff and leaching of nutrients and herbicides to waterways (e.g., World Heritage-listed Great Barrier Reef catchments) cannot be neglected (Cherubin et al., 2016; Liu et al., 2018c; Duhan et al., 2020).

Microorganisms have a major role in soil functioning, and their disturbance (e.g., by cultivation and other land management practices) can lead to detrimental effects on ecosystem processes and services (Tosi et al., 2016). The dynamic interactions between soil microbial community composition and functions, and soil C and N pools are key to better understanding of long-term changes in soil quality and health following disturbance. In addition, conversion of natural ecosystems to agricultural lands would result in alteration of soil C and N cycles through soil organic matter depletion and loss of labile fractions of soil nutrients (Cherubin et al., 2016; Guimaraes et al., 2018; Liu et al., 2018a), along with a reduction in soil microbial biomass, basal respiration, and enzymatic activities (Brackin et al., 2014; Tosi et al., 2016). The impacts of land use change on soil physicochemical properties

and soil C stocks and turnover have been extensively investigated, however there is still a significant knowledge gap regarding the potential changes in bioavailability of soil N pools and the abundance of N cycling functional genes following long-term conversion of native forests and grasslands to sugarcane systems in tropical soils. Therefore, the main objectives of this study were to assess the long-term effects of land use change in a humid tropical climate, from native forest and pasture to sugarcane, on (1) soil C and N cycling and bioavailability (to plants and microorganism); and (2) functionality and abundance of soil N cycling genes. The underlying hypotheses were: (a) land use change would decrease total organic C (TOC) and N (TN) stocks and their bioavailability; and (b) land use change would reduce the abundance of soil microbial communities in sugarcane cultivations compared with adjacent native forest or pasture.

2. Materials and Methods

2.1 Site description and soil sampling

The experimental site was in Freshwater (16° 52′ 59″ S, 145° 42′ 19″ E; 19 m above sea level), near Cairns, Queensland, Australia. The area has tropical climate with annual mean rainfall of around 2000 mm and mean maximum and minimum temperatures of 29.1°C and 20.8°C, respectively. To assess the impacts of land use change on soil physicochemical and biological properties, two adjacent paired sites (native forest vs sugarcane cropping for 78 years; pasture for 78 years vs sugarcane cropping for 78 years) were selected. Five paired (less than 50 m distance) composite surface soil samples (0-10 cm depth; 15 cores were bulked for each composite sample) were collected from each of the paired land uses. The collected fresh soil samples were passed through a 2 mm sieve and divided into three parts: 1) stored at -20°C for molecular analyses within two weeks of sampling; 2) stored at 4°C for biological analyses within two weeks of sampling; and 3) air-dried prior to physicochemical

analyses. The results were expressed on an oven-dry weight basis. For measurement of bulk density (BD), undisturbed cores (10 cm diameter; top 10 cm soil; 5 replicates at each of the 4 locations) were taken.

In the present study, it was assumed that the adjacent paired sites (under different land uses) were similar in their origin and parent materials. This assumption has been generally accepted and applied as the basis of many paired site studies (Chen et al., 2004; Liu et al., 2021). The adjacent paired sites were also uniform due to their long-term management history (78 years). We acknowledge the limitation of this study, as for many other paired site studies, conducted using pseudo-replication.

2.2 Soil physicochemical analysis

Soil particle size distribution (soil texture) was measured by Low Angle Laser Light Scattering (LALLS) using a laser diffraction particle size analyser Master sizer 3000 (Malvern Instruments). Soil bulk density was measured by oven-drying and weighing of collected undisturbed cores. Soil pH and EC were measured in a 1:5 (soil:distilled water) suspension using a glass electrode and EC meter (Rayment and Lyons, 2011). Soil total OC and N contents were measured by dry combustion (at 1200°C) using a LECO CN analyser (TruMac NO. 830-300-400). Total soil P content was measured using an inductively coupled plasma optical emission spectrometer (ICP-OES; Perkin Elmer; Optima 8300) following digestion in a mixture of nitric and perchloric acids (Miller, 1998). Soil ammonium (NH_4^+-N) and nitrate (NO_3^--N) concentrations were measured using a SEAL AA3 Continuous Segmented Flow Analyzer (SEAL Analytical Limited. USA) after extraction by 2M KCl (1:5 suspension ratio) and filtration of the extracts through Whatman 42 filter paper (Rezaei Rashti et al., 2015). The concentration of dissolved organic C (DOC) and N (DON) were

measured using a TOC/N analyser (Shimadzu TOC-VCSH/CSN) after extraction of a 1:5 (soil:distilled water) suspension and filtration with a non-pyrogenic 0.45 μm syringe filter.

2.3 Soil biological analysis

Soil microbial biomass C (MBC) and N (MBN) contents were determined using the chloroform fumigation-extraction method. Both non-fumigated and chloroform fumigated fresh soil samples were extracted with 0.5 M potassium sulphate (K_2SO_4), filtered through a Whatman 42 filter paper, and analysed using a TOC/N analyser (Shimadzu TOC-VCSH/CSN). The MBC and MBN contents were calculated as the difference in DOC and DON between non-fumigated and chloroform-fumigated samples, respectively, multiplied by a factor of 2.64 for MBC and 2.22 for MBN (Rezaei Rashti et al., 2019).

Soil respiration was measured using a 35-day incubation procedure as described by Liu et al. (2018b). The fresh soil samples (25 g oven-dry equivalent) were incubated at 22°C and 60% of water holding capacity (WHC) under aerobic condition in a sealed glass jar. The CO_2 produced during the incubation process was trapped in a 1.0 M NaOH solution and measured by acid back-titration (0.1 M HCl) at 1, 3, 7, 14, 21, 28 and 35 days after start of the incubation. The bioavailable C content of each soil sample was estimated using cumulative production of CO_2 during the entire incubation period. The microbial metabolic quotient (qCO_2) value was calculated as the ratio of respired (bioavailable) C to MBC content (Liu et al., 2023).

Soil DNA was extracted from 0.5 g samples using the MoBio Powersoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) as described by Liu et al. (2013). The quality and quantity of the extracted DNA were determined using a Nanodrop spectrophotometer (Thermo Fisher scientific, Massachusetts, USA) at 260 nm and the ratios of A260/280 ranged from 1.8 to 2.0. The polymerase chain reaction (PCR) fragments of microbial functional

genes were amplified using primers and thermal conditions described by Lan et al. (2019). The purified PCR products were cloned into the TOPO TA cloning vector (Invitrogen, Carlsbad, CA, USA). The Qiagen Miniprep kit (Qiagen, Germantown, MD, USA) was used to extract plasmids from positive clones for making standard curves for quantitative analysis. The investigated microbial genes were *16S rRNA* gene, *amoA* gene for ammonia-oxidizing archaea (*AOA*) and ammonia oxidizing bacteria (*AOB*), nitrate reductase gene (*narG*), nitrite reductase genes (*nirK* and *nirS*), nitrous oxide reductase gene (*nosZ*) and alkaline phosphatase gene (*ALP*). The reactions were conducted on the Mastercycler® ep realplex real-time PCR system (Effendorf, Hamburg, Germany) in three replicates. Melting curves and agarose gel running of PCR products at the end of each quantitative real-time PCR were used to check amplification specificity. No template controls gave null or negligible values. The estimation of PCR inhibitors in the DNA extracted solution using a 1:10 soil DNA dilution showed no inhibition for PCR process in the analysed soil samples.

2.4 Data processing and statistical analysis

To assess the significance of differences in soil physicochemical and biological properties between different land uses, statistical analysis was performed by Independent-Samples T Test using the IBM SPSS Statistics 29 software package (IBM SPSS Statistics Version 29.0. Ar-monk, NY: IBM Corp.). The differences at $P \leq 0.05$ between different land uses, using LSD test, were considered statistically significant and all variables were tested for normality of distribution using Kolmogorov-Smirnov test. The copy numbers of the microbial functional genes were log-transformed and centred. Principal component analysis (PCA) was conducted using IBM SPSS Statistics 29 software package to differentiate land uses based on their biochemical properties. The PCA was performed on the correlation matrix to eliminate the effect of numerical differences and measurement units among the variables.

3. Results

3.1 Soil physicochemical properties

The sugarcane sites had significantly higher clay content and lower sand content than the native forest and pasture land uses (Table 1). In the pasture pair, BD was lower in the sugarcane, but in the forest pair, BD was higher in the sugarcane ($P \leq 0.05$). Sugarcane soil had significantly higher pH and lower EC than native forest and pasture soils. Total OC contents of sugarcane soils were approximately half that of native forest and pasture soils, and WHC was also substantially lower ($P \leq 0.05$) in sugarcane than native forest and pasture. Similar differences between land uses were observed with total N content, and C:N ratio was higher in sugarcane than native forest and pasture lands ($P \leq 0.05$). Sugarcane soils also had lower total P content, lower C:P ratio and lower N:P ratio than the other land uses.

3.2 Soil bioavailable carbon and nitrogen contents

Mineral N contents (NH_4^+-N + NO_3^--N) were lower in sugarcane soils than native forest and pasture soils, with the difference being due to higher NH_4^+-N in pasture and higher NO_3^--N in forest ($P \leq 0.05$; Table 2). The sugarcane soils had similar DON concentration to forest and lower DON concentration than pasture soils. However, DON:TDN ratios were significantly higher in sugarcane soils than other land uses. The DOC contents in sugarcane soils were also significantly lower than other land uses, with bigger differences observed between sugarcane and pasture than sugarcane and native forest. Consequently, no significant differences were observed between DOC:DON ratios in different land uses. The bioavailable carbon contents, measured as cumulative CO_2-C emission over a 35-day aerobic incubation period, indicated significantly lower values in sugarcane soils than pasture and native forest soils (Fig. 1). The difference in aerobic respiration was apparent in the first 7 days of incubation and increased over time by the end of incubation. This observation indicated lower aerobic microbial

activity in investigated sugarcane soils than other land uses under optimum temperature and moisture contents.

3.3 Soil biological properties and abundance of microbial functional genes

Sugarcane soils had significantly lower MBC and MBN contents than native forest and pasture land use, with bigger differences between sugarcane and pasture than sugarcane and native forest (Table 3). The MBC:MBN ratios in sugarcane soils were generally lower and higher than pasture and native forest soils, respectively. A significant reduction in percentage of MBC:TC and MBN:TN were observed in sugarcane in comparison with pasture land use, however there was no such significant difference between sugarcane and native forest soils. The aerobic respiration rates were also significantly lower in sugarcane soils than native forest and pasture soils, with the highest respiration rates observed in native forest land use. The estimated qCO_2 value for sugarcane soils were slightly higher than native forest, and significantly higher than pasture system, indicating lower efficiency of soil microbial functional activity in sugarcane land use compared with other land uses.

The 16S rRNA gene abundance was significantly lower in sugarcane soil than pasture soil, however no significant differences were observed in the *amoA* gene copy numbers for ammonia-oxidizing bacteria (*AOB*) and archaea (*AOA*) between these land uses ($P > 0.05$; Table 4). The abundance of *AOA* was also significantly lower in sugarcane than native forest soil, while no significant differences were observed in the 16S rRNA and *AOB* gene copy numbers between these soils. Among the N cycling associated genes, only *narG* showed significantly higher abundance in sugarcane than pasture land use, while *narG*, *nosZ*, *nirK* and nirS genes had significantly lower copy numbers in sugarcane soil compared with native forest soil. In addition, the abundance of *ALP* was significantly lower in sugarcane than other land uses.

The principal component analysis (PCA) showed that sugarcane sites can be clearly separated from the native forest and pasture sites along the first two principal components, which accounted for 74.4% of the total variance in biochemical properties (Fig. 2). The first principal component (PC1; total variance of 43.9%) strongly separated sugarcane from pasture soil, with forest soil intermediate. The second component (PC2; total variance of 30.5%) mainly discriminated between native forest and the other two systems. The PC1 was most strongly correlated (≥ 0.9) with TOC, TN, MBC and MBN contents, while PC2 was most strongly correlated (≥ 0.9) with NO_3^--N content and abundance of N and P cycling functional genes (i.e., *nosZ*, *nirK*, *nirS*, and *ALP*).

4. Discussion

4.1 Effect of land use change on soil physicochemical properties

Long-term conversion from pasture and native forest to sugarcane decreased ($P \leq 0.05$) soil TOC (45% - 48%), TN (51% - 54%), and TP (26% - 37%) pools, indicating an overall soil degradation following sugarcane cultivation in tropical climate of this experiment. However, soil pH increased, presumably due to regular application of lime. Tropical soils are generally old and highly weathered (i.e., acidic, low nutrient contents, high iron and aluminium oxides, and high P immobilisation capacity), so soil P bioavailability may be used as a biochemical indicator of quality and function, with reduction of soil P stock indicating degradation and loss of productivity (Stone and Plante, 2014; Cherubin et al., 2016; Nasto et al., 2017; Leite et al., 2018). Depletion of soil P pools in tropical agricultural systems can be related to accelerated soil organic matter decomposition (≈ 50% of soil P pools is contained in organic matter) due to structural degradation, and nutrient export with crop yield (Kotowska et al., 2015; Maranguit et al., 2017). Liu et al. (2018a) observed a significant reduction in TOC (13.6%), TN (17.3%), and TP (8.5%) stocks in topsoil (0-30 cm), 27 years after conversion

from pasture to cropland in a temperate semiarid continental climate in northwest China. Guimaraes et al. (2018) also reported a decrease of 46% in soil TOC content (0-20 cm) 19 years after land use conversion from native forest to sugarcane in a humid subtropical climate in Brazil.

It has been reported that the changes in soil C stocks after land use conversion is very slow, and the effects of different management practices on the quality and quantity of soil C pools would occur over several decades (Poeplau et al., 2011) as the soil C cycle reaches a new equilibrium. Total and dissolved OC contents in both sugarcane sites in this study, were significantly lower than the values in the pasture and native forest soils, 78 years after land use conversion (Tables 1 and 2). This may be attributed to tillage (resulting in promotion of organic matter oxidation by oxygen diffusion into soil profile and enhancement of aerobic respiration process following soil aggregate breakdown), high rate of organic matter decomposition, and downward transport of dissolved C during high rainfall seasons in tropical sugarcane systems (De Figueiredo et al., 2015; De Oliveira Bordonal et al., 2017) after a long-term land use conversion. Well-developed root system and higher stability of soil structure in pasture lands (DuPont et al., 2010; Dos Santos et al., 2019) and native forests would also result in higher soil C sequestration and labile OC accumulation in these land uses, compared to agricultural lands (Bordonal et al., 2017). Our results also indicated a similar trend in breakdown and release of labile OC and ON in paired land uses of this experiment, as there were no significant differences in DOC:DON ratios following long-term conversion of pasture and native forest to sugarcane. Mello et al. (2014) and Franco et al. (2015) reported similar observations following long-term land use conversion (≈ 20 years) from native vegetation and pasture to sugarcane in tropical climate. This would indicate that maintenance of sugarcane residues on soil surface (i.e., trash blanket practice) would not improve long-term C sequestration and reverse the increased mineralisation rate of soil C,

induced by disturbance of soil profile, following conversion of pasture and native forest into sugarcane, especially in tropical climate of this study with high annual rainfall and temperature. Tosi et al. (2016) and De Oliveira Bordonal et al. (2017) also indicated the important role of climate (i.e., high temperature and soil water content) in acceleration of soil OC mineralisation and loss following agricultural disturbance.

Soil OC plays an important role in soil aggregation and water holding capacity (Liu et al., 2011), which agrees with a significant decline of water holding capacity in sugarcane (with significantly higher clay contents) compared with pasture and native forest soils in this study. In addition, mineralisation rate of labile OC is associated with release of micro- and macro-nutrients in soil profile (Da Silva Oliveira et al., 2017) and significantly contributes to nutrient bioavailability and cycling, as well as microbial and plant biomass production. Soil C:N:P ratios (as one of the main indices of soil quality and fertility) in this experiment indicated a higher reduction in total N content compared with total C and P contents following land use change to sugarcane (Table 1). It is reported that indigenous soil N supply plays a crucial role in maintaining crop productivity in agricultural lands, as about half of the N uptake in these systems is expected to come from mineralisation of soil organic matter (Luce et al., 2014; Li et al., 2018). This agrees with the observed N limitation for microbial biomass production in sugarcane soils of this study (Table 3), as soil microbial demand for C and N is regulated by the changes in the stoichiometry of elemental resources for microbial consumption induced by land use conversion. Brackin et al. (2014) also reported that N bioavailability was the main limiting factor for soil microbial activities in an Australian subtropical sugarcane soil, while both P and N limitations were observed in microbial communities of adjacent forest ecosystem.

4.2 Effect of land use change on soil biological properties and microbial functional genes

Soil microbial composition and functionality are the most sensitive indicators for assessing the changes in land use and management (Miura et al., 2016), with general reduction in soil microbial biomass, respiration and enzymatic activities reported following conversion of non-cultivated lands to agricultural systems (Tosi et al., 2016). Different land uses may vary in the quality and quantity of their OC, with pristine forest vegetation usually dominated by recalcitrant C substrates, while agricultural lands contain more labile OC which can decompose faster by soil microorganisms. Changes in decomposition rates of soil organic C, due to land use conversion, may significantly alter soil microbial growth and functionality towards the dominance of well-adapted microbial species. This is mainly governed by changes in content and bioavailability of soil labile carbon (DOC), which is the main C and energy source for soil microorganisms (Batlle-Bayer et al., 2010; Vinhal-Freitas et al., 2017, Yao et al., 2019). Consequently, soil microbial biomass, as the most active component of soil organic matter, can be used as a sensitive indicator for prediction of changes in soil biogeochemical cycles and C and nutrient flows in soil profile (Kaschuk et al., 2010; Tosi et al., 2016). This study showed a reduction in soil microbial biomass (60%), soil respiration (49%) and abundance of nitrogen cycling functional genes in sugarcane compared with their adjacent pasture and native forest soils (Tables 3 and 4, and Fig. 1). This would indicate a significant decline in amount, activity, and diversity of soil microbial community, as well as the overall biological health of investigated sugarcane soils after land use conversion.

Soil microbial respiration is an indicator of soil OC mineralisation and is usually associated with soil microbial biomass content. In a soil profile with balanced physicochemical properties, soil microorganisms are under minimum environmental stress and can freely access soil OC as their food and energy source through aerobic respiration process. However, intensive cultivation and disturbance of soil profile would result in generation of environmental stress for soil microbial communities and consequently reduce the efficiency

of carbon use (i.e., higher metabolic quotient (qCO_2) values) by soil microorganisms. This is consistent with the findings of this study that showed lower qCO_2 values and higher C use efficiency (i.e., lower respiration in relation to microbial biomass content) in pasture and native forest sites compared with their adjacent sugarcane lands (Table 3). Vinhal-Freitas et al. (2017) also reported an increase in qCO_2 values in two sugarcane systems following land use conversion from pasture and native cerrado systems in a tropical climate in southwestern Brazil. They related this observation to high disturbance of soil profile during cultivation of sugarcane and consequent inefficiency of carbon use by soil microorganisms in this agroecosystem.

Assessing the effect of land use conversion on functional potential of soil microbial community would help to improve our understanding of how these systems adapt to changes in environmental conditions and predict their ecosystem services and activity levels under current and future stressed environments. Several studies indicated significant differences in composition and functionality of soil microbial communities between agricultural and native forest land uses (Mendes et al., 2015; Wood et al., 2017; Ding et al., 2022). Changes in soil microbial community composition, induced by land use conversion, would result in alteration of microbial functional potential in ecosystems with limited biodiversity, while in resilient systems soil microbial communities can exhibit high functional redundancy to environmental stresses (Louca et al. 2018). Nitrogen cycling process is one of the most important ecosystem functions (both natural and agricultural ecosystem) mediated by soil microorganisms and can be affected by changes in land management practices following land use conversion (Hermnas et al., 2020). Nitrification process is mediated by ammonia-oxidising bacteria (*AOB*) and archaea (*AOA*) and catalysed by ammonia monooxygenase enzyme (Prosser et al., 2020). However, denitrification process is regulated by a variety of bacteria and catalysed by a set of enzymes encoded by distinct functional genes, such as nitrate reductase (*narG*;

reduction of NO_3^- to NO_2^-), nitrite reductases (*nirK* and *nirS*; reduction of NO_2^- to NO), and nitrous oxide reductase (*nosZ*; reduction of N_2O to N_2) (Jones et al., 2013). Quantification of these functional genes can illustrate the association of soil microbial nitrifiers and denitrifiers with N cycling processes in different land uses. This study showed a general reduction in abundance of N cycling functional genes following conversion of native forest to sugarcane, while the opposite pattern observed in sugarcane land converted from pasture. This shift within the abundance of N cycling functional genes can be related to the alteration of soil physicochemical properties such as mineral N content, labile organic C and N contents, pH value, and partial oxygen pressure in soil micropores (Zhang et al., 2012; Song et al., 2019) following changes in lang use management. Our results also indicated the functional redundancy of nitrification process (oxidation of NH_4^+ to NO_3^- mediated by *AOA* and *AOB*) following long-term land use conversion in both sugarcane sites, while this was only the case for denitrification process (reduction of NO_3^- to N_2 mediated by *narG*, *nosZ*, *nirK*, and *nirS*) in sugarcane converted from pasture (Table 4). The decrease in the abundance of denitrification genes would indicate a reduction of microbial denitrification potential in sugarcane soil following land use conversion from native forest. It is suggested that the degree of soil microbial functional redundancy to land use change would be regulated by the magnitude of taxonomic differences between sites (Bell et al., 2005), and the differences in their biochemical properties (Louca et al., 2016) such as nutrient bioavailability and creation of aerobic/anaerobic microsites.

Conclusions

This study assessed the long-term effects of land use conversion, from native forest and pasture cropping to sugarcane, on soil biogeochemical properties, C and N cycles, and related microbial functional processes. Results shown a reduction in soil C, N, and P pools in both sugarcane sites, following land use conversion, with N bioavailability as the main limiting

factor for soil microbial activities. A general reduction was observed in abundance of N cycling functional genes following conversion of native forest to sugarcane, while sugarcane converted from pasture showed an opposite pattern. The results also indicated the functional redundancy of nitrification process (bacterial *amoA*, archarel *amoA*) following land use conversion in both sugarcane sites, while this was only the case for denitrification process (*nosZ*, *nirK*, *nirS*) in sugarcane converted from pasture.

Acknowledgments

This project was financially supported by the Griffith University-James Cook University Collaborative Research Fund. The authors would like to thank the farmer Mark Savina for providing access to the sampling sites.

Statements and Declarations

Conflict of interest: The authors have no relevant financial or non-financial interests to disclose. The authors have no competing interests to declare that are relevant to the content of this article. All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript. The authors have no financial or proprietary interests in any material discussed in this article.

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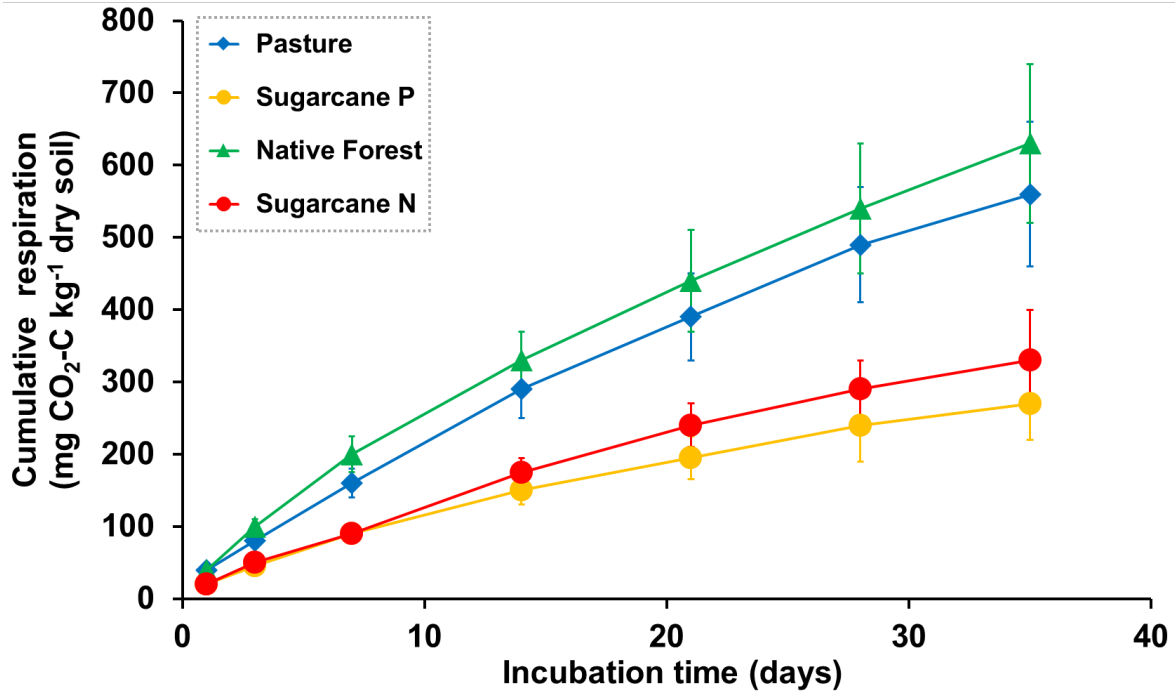

Fig. 1: Effect of land use change on soil bioavailable carbon content. Sugarcane P = sugarcane site converted from pasture land use; Sugarcane N = sugarcane site converted from native forest land use.

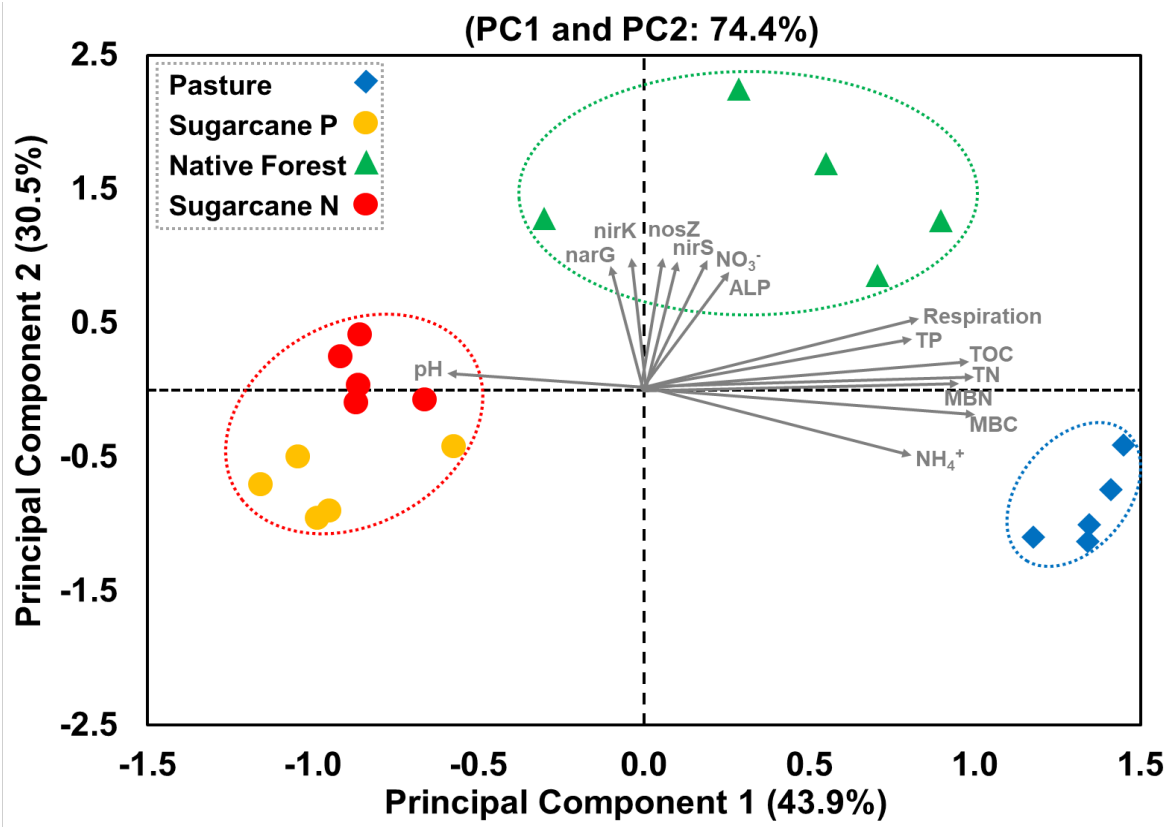

Fig. 2: Ordination plot of Principal Component Analysis (PCA) loading values of the first two principal components (PC) for differentiating land uses based on soil biochemical properties (n = 5). Numbers in parentheses are percentage variance by each principal component. Sugarcane P = sugarcane site converted from pasture land use; Sugarcane N = sugarcane site converted from native forest land use.

Table 1: Selected physicochemical properties of soils under different land uses

Land use	pH (1:5 water)	EC ($\mu S\ m^{-1}$)	BD ($g\ cm^{-3}$)	WHC (%)	Total OC ($g\ kg^{-1}$)	Total N ($g\ kg^{-1}$)	Total P ($mg\ kg^{-1}$)	C:N ratio	C:P ratio	N:P ratio	Sand (%)	Clay (%)	Silt (%)
Pasture	5.30	42.8	0.99	64.2	22.86	2.32	484	9.9	47.2	4.8	30.6	19.4	50.0
Sugarcane P	5.85	31.7	0.71	48.8	11.85	1.07	359	11.1	33.1	3.0	15.8	31.4	52.8
P (one tail)	0.017	0.040	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	0.144
Native forest	5.55	74.5	0.90	53.9	21.68	1.93	534	11.2	40.4	3.6	42.4	15.2	42.4
Sugarcane N	6.14	45.7	0.94	44.4	11.95	0.95	336	12.6	35.6	2.8	33.0	21.8	45.2
P (one tail)	0.002	0.010	<0.001	<0.001	0.001	0.001	<0.001	0.001	0.006	0.003	<0.001	<0.001	0.086

Sugarcane P = sugarcane site converted from pasture land use; Sugarcane N = sugarcane site converted from native forest land use; EC = electrical conductivity; BD = bulk density; WHC = water holding capacity. The reported data are means of 5 replicates.

Table 2: Effects of sugarcane cropping on concentration of soil mineral nitrogen, soluble organic nitrogen, and soluble organic carbon

Land use	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	DON (mg kg^{-1})	DON:TDN (%)	DOC (mg kg^{-1})	DOC:DON ratio
Pasture	18.2	3.8	15.2	40.8	113.6	7.6
Sugarcane P	7.6	4.0	8.9	43.2	68.7	7.8
P (one tail)	<0.001	0.405	<0.001	0.014	0.001	0.282
Native forest	7.9	16.1	10.0	28.6	95.2	10.2
Sugarcane N	8.0	6.6	9.2	38.0	76.0	8.9
P (one tail)	0.469	0.003	0.269	0.011	0.034	0.244

Sugarcane P = sugarcane site converted from pasture land use; Sugarcane N = sugarcane site converted from native forest land use; DON = dissolved organic nitrogen; TN = total dissolved nitrogen; DOC = dissolved organic carbon; TDN = (NH_4^+-N + NO_3^--N + DON). The reported data are means of 5 replicates.

Table 3: Selected biological properties of soils under different land uses

Land use	MBC (mg kg^{-1})	MBN (mg kg^{-1})	Microbial C:N ratio	MBC:TOC (%)	MBN:TN (%)	Respiration rate (µg CO_2-C, g^{-1} h^{-1}, 7 days)	qCO_2 (µg CO_2-C mg^{-1} MBC h^{-1})
Pasture	571.8	82.8	6.9	2.50	3.57	0.97	1.73
Sugarcane P	181.6	29.1	6.2	1.53	2.73	0.54	3.13
P (one tail)	<0.001	<0.001	0.015	0.004	0.048	0.006	0.015
Native forest	395.0	65.4	6.0	1.83	3.42	1.18	2.99
Sugarcane N	189.6	27.9	6.8	1.58	2.79	0.59	3.17
P (one tail)	0.002	0.001	0.146	0.131	0.240	0.019	0.374

Sugarcane P = sugarcane site converted from pasture land use; Sugarcane N = sugarcane site converted from native forest land use; MBC = Microbial biomass C; MBN = Microbial biomass N; TOC = Total organic C; TN = Total N; qCO_2 = Metabolic quotient. The reported data are means of 5 replicates.

Table 4: Effects of sugarcane plantation on the abundance of nitrogen and phosphorus associated soil microbial functional genes

Land use	*16S RNA gene*	*AOB*	*AOA*	*narG*	*nosZ*	*nirK*	*nirS*	*nirK:nosZ*	*narG:nirK*	*ALP*
Pasture	9.515	4.920	6.047	6.939	4.603	4.735	7.043	1.030	1.467	6.737
Sugarcane P	9.321	5.327	6.162	7.274	4.688	4.871	6.971	1.041	1.493	6.567
P (one tail)	0.019	0.069	0.309	<0.001	0.337	0.108	0.316	0.372	0.176	0.028
Native forest	9.447	5.443	7.098	7.787	5.969	5.703	7.940	0.956	1.368	7.570
Sugarcane N	9.400	5.248	6.361	7.157	4.989	5.079	7.497	1.018	1.410	6.796
P (one tail)	0.448	0.178	0.001	0.007	0.005	0.019	0.020	<0.001	0.124	<0.001

Sugarcane P = sugarcane site converted from pasture land use; Sugarcane N = sugarcane site converted from native forest land use; *AOB* = ammonia-oxidizing bacteria; *AOA* = ammonia-oxidizing archaea; *narG* = nitrate reductase gene; *nosZ* = nitrous oxide reductase gene; *nirK* = nitrite reductase gene; *nirS* = nitrite reductase gene; *ALP* = alkaline phosphatase. The values are reported based on log gene copy numbers g^{-1} dry soil. The reported data are means of 5 replicates.