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The influence of land use on nitrate leaching after flooding in a sugarcane catchment

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Cover photo: Flooded *Melaleuca* adjacent to sugarcane.

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Contents

1.0 Executive Summary

1. Payment for ecosystem service (PES) schemes make payments to those carrying out ecosystem restorations or improved land management practices that provide desired benefits, such as water quality improvements, carbon sequestration, flood regulation, water security or habitat for wild resources. Tailoring restoration projects to the desired outcomes of PES schemes provides an avenue for funding restoration. While vegetative restoration projects are increasingly attracting payments for their carbon sequestration benefits, the benefits to water quality improvement from floodplain reforestation has received little attention. Reforested floodplains can remove nitrogen delivered from floods through vegetation sequestration and through denitrification. The latter occurs through denitrifying bacteria decomposing leaf litter and associated organic matter as nitrogen-laden water leaches through the soil column.
2. Greening Australia recently converted an old sugarcane field into approximately 4 ha of *Melaleuca* plantings as a coastal floodplain site, adjacent to Palm Creek, near Forrest Beach in north Queensland. Adjacent to the recently planted field are other distinct fields containing either weedy grass, mature *Melaleuca* trees (planted ~30 years ago), and actively farmed sugarcane. The site is also known to flood with water draining upstream sugarcane, likely resulting in nitrate deposition across all sites. This provided an ideal case study location to examine the influence of different vegetation cover on nitrate leaching.
3. Over a 12-month period, between December 2021 and 2022, nitrate leaching across the different land uses was assessed using the ion-exchange resin method. It was found that the sugarcane site leached approximately 17 kg N/ha (congruent with literature estimates), which was substantially greater than the grass site, mature *Melaleuca* site, and the recently restored *Melaleuca* site, of which all three leached <1 kg N/ha. This indicates that retiring land from low-lying sugarcane can essentially eliminate nitrate leaching altogether. However, in this instance flooding did not occur and no nitrate from the catchment could have been deposited and removed.
4. This study also experimentally examined whether nitrate leaching rates would differ with different application levels of nitrate between sugarcane and mature *Melaleuca*, simulating different applications occurring from floodwaters during the onset of the wet season. Nitrate applied experimentally to soils beneath sugarcane and mature *Melaleuca* leached considerably in the sugarcane but not in the mature *Melaleuca*. It is hypothesized that the high soil carbon in the mature *Melaleuca* site likely enabled greater microbial growth and immobilization of inorganic nitrate, thus reducing leaching.
5. Metabarcoding of soil microbial assemblages indicates that all four adjacent land uses had very low proportions of nitrate reducing bacteria, likely arising from a lack a persistent nitrate (at high levels) and anoxic conditions. As a result, short term deliveries of nitrate are unlikely to be reduced as the populations required to reduce nitrate are not maintained in between events. Furthermore, the nitrate application experiment also indicated that the Shannon's Diversity of Nitrate Reducing bacteria was positively correlated with nitrate application rates, regardless of vegetation type, suggesting it may be a useful indicator of soil nitrogen dynamics in future.
6. It is recommended that future studies scope locations where planting can occur to ensure vegetation is regularly inundated by nitrate-laden waters, and the efficacy of multispecies plantings to bolster denitrification capacity is explored.

2.0 Introduction

Wetlands are vital for humanity and include some of the most productive, diverse, and service-rich ecosystems in the world (Gardner and Finlayson, 2018). Ecosystem services are the benefits to humans that nature provides for free (Costanza et al., 1997, 2014). Services provided by wetlands include food production (e.g., fish, birds and vegetables), protection from flooding and storm surge inundation, provision of clean water and climate stability, and timber resources for construction (Millennum Ecosystem Assessment, 2005; Mitsch et al., 2015). In 2011, swamp wetlands were estimated to provide services valued at, on average, $25,681 USD/ha/yr (Costanza et al., 2014; Kubiszewski et al., 2020). Despite their high value, wetlands have faced substantial drainage in lieu of urban and agricultural development, with an estimated 87% decline in natural wetland extent since pre-industrial times (Davidson, 2014; Antonio Ballut-Dajud et al., 2022). Of those that remain, approximately 89% of wetlands globally are unprotected (Reis et al., 2017), and often continue to be impacted by water abstraction, eutrophication, grazing, and climate change. For example, a sea level rise of 50 cm by 2100 is estimated to drive the loss 46-59% of global coastal wetlands, with a rise of 110 cm predicted to increase losses to ~78% (Spencer et al., 2016). Halting and reversing this decline will be necessary if we are to progress in achieving the United Nations Sustainable Development Goals, particularly the provision of clean water, sustainable food and resource security, climate security and biodiversity conservation (United Nations General Assembly, 2015; Gardner and Finlayson, 2018; United Nations, 2020).

Wetlands in Australia also follow the global pattern of decline, with swamps being those most extensively drained (Davis and Froend, 1999; Finlayson and Rea, 1999; Canning and Waltham, 2021). Within Australia's Great Barrier Reef (GBR) catchment, despite coastal wetlands providing critical habitat that supports the life-cycle of migratory fish occupying the reef to be completed and treating catchment runoff to the reef, considerable areas of wetlands have been lost. While much of this occurred shortly after European settlement, between 2001 and 2017 there was a loss of 7688 ha of wetlands across the catchment. Recent efforts, however, have seen increased wetland protection policy and restoration ambitions across the nation (Max Finlayson, 2018; Adame et al., 2019; Creighton et al., 2019), largely driven by a desire for their ecosystem services (Matzek et al., 2019).

Payment for ecosystem service (PES) schemes make payments to those carrying out ecosystem restorations that provide desired benefits, such as water quality improvements, carbon sequestration, flood regulation, water security or habitat for wild resources (Farley and Costanza, 2010; Salzman et al., 2018; Canning et al., 2021). Of national interest, the Australian Government recently released a statutory method for assessing and awarding carbon trading credits, under the Emissions Reduction Fund, for 'blue carbon' projects focused on restoring coastal wetlands, such as mangrove forests and coastal tree swamps (Macreadie et al., 2017; Costa et al., 2020). In Queensland, the Queensland Government's Land Restoration Fund (LRF) seeks to pay a premium price for carbon credits from restoration projects that not only sequester carbon but also demonstrate enhanced delivery of other ecosystem services. Within Australia's Great Barrier Reef (GBR) catchment, the Reef Credit Scheme is emerging, with aims to make payments for actions, such as wetland restorations, that deliver water quality benefits to the downstream Great Barrier Reef – a World Heritage Site and Federal Marine Park (Eco-Markets Australia, 2020). In a GBR catchment and in the Murray-Darling Basin, PES schemes have also financially supported wetland restorations for their hydrological benefits (Connell and Grafton, 2011; Canning et al., 2021). Payments from single PES schemes may not always render a wetland restoration financially viable, increasing interest in bundling payments from multiple schemes to reward the provision of multiple services may improve financial viability (Robertson et al., 2014; Canning et al., 2021; Costanza et al., 2021). While the Australian Government's Emissions Reduction

Fund can provide payments for carbon sequestration benefits by forest and coastal ecosystem restoration, the benefits to water quality improvement from floodplain reforestation has received little attention. Reforested floodplains can remove nitrogen delivered from floods through vegetation sequestration and through denitrification. The latter occurs through denitrifying bacteria decomposing leaf litter and associated organic matter as nitrogen-laden water leaches through the soil column. These mechanisms being well described in riparian buffer vegetation literature (Zhang et al., 2010; Lind et al., 2019; Lyu et al., 2021), and the effectiveness of buffers to provide these benefits is dependent on buffer width, buffer slope, soil type, vegetation composition, and the size of impact to be mitigated. However, the lack of data on quantifiable water quality improvement benefits has been a major barrier to receiving payments for this benefit.

Nitrate transport from land through to aquatic systems is highly variable throughout the year, largely dependent on accumulation within soil, rainfall, and agricultural practices (e.g., fertiliser application and irrigation). Within the sugarcane areas of Australia's Great Barrier Reef catchment, which experiences a tropical wet-dry climate, most nitrate is transported to the reef as large pulses or floods at the beginning of the wet season. Throughout the year, nitrate accumulates within agricultural soils and below the crop root zone which is then heavily mobilised in the first few large rain events of the wet season, which often cause flooding. Much of the nitrate load carried within floodwaters will flow to the coast; however, some of it will be deposited on floodplains where some of it will be attenuated by vegetation and microbial processes – preventing it from flowing to the coast. There is evidence from the literature on riparian buffers suggests that buffers with forest have significantly greater denitrification and plant nitrogen removal rates than grass. However, the efficacy of plant removal decreases with time as the plants reach maturity and growth slows. Once plants reach maturity, nitrogen removal primarily occurs by microbial-driven denitrification, which is promoted by increased soil carbon from leaf litter and root exudates (Zhang et al., 2010; Lyu et al., 2021). Aside the from the influence of land slope and soil type, vegetation type is highly influential on the effectiveness of nitrate removal, and it is anticipated that this will also be true in floodplains. Investigating the rates of nitrate leaching from the root zones across different floodplain vegetations allows the identification of vegetation that results in the least leaching. Future revegetation programs can then tailor plantings towards vegetation that is effective in removing nitrate from floodwaters and potentially attract payments for this benefit, in addition to the benefits the plantings could provide for climate change, recreation and biodiversity.

Investigating and predicting nitrate leaching from different vegetation types within floodplains is a challenging task, particularly if conventional methods that require automated drainage lysimeters are used, given the unpredictable nature of the timing, depth, coverage and depth of floodwaters (Qian and Schoenau, 2011; Willich and Buerkert, 2016; Grahmann et al., 2018). Not only would there be practical constraints of inhibited access to collect samples but there would also be a high likelihood of in-situ sensor destruction. To circumvent access and sensor destruction constraints, ion-exchange resins present a novel and cost-effective way of passively measuring nutrient loss over time by adsorbing nitrate ions as they leach through the soil column. Ion-exchange resins are being used increasingly in both agricultural and ecological studies to assess nitrate leaching, and they have been shown effective compared with traditional lysimeter methods. As resins sample passively, they are also able to reflect conditions between sampling periods, which can be highly variable as land use practices, plant growth and rainfall are variable (Qian and Schoenau, 2011). We consider that this approach would be an ideal candidate as a cost-effective, informative, easily scalable, low risk and standardisable methodology for directly assessing nitrate leaching in floodplains.

As a supplement to the ion-exchange resin methods of assessing nitrate leaching, assessments of soil microbial activity, traits and assemblages, could also provide an indicator of nitrate removal efficacy (Pommier et al., 2018). Soil microbes represent a large powerhouse of organisms that can both increase and reduce nitrate in soils. The presence of denitrifying bacteria – those that metabolize nitrate into nitrous oxide and nitrogen in hypoxic condition – can indicate the extent to which there are favourable conditions for nitrate to be metabolized (via denitrification) and prevented from leaching. For example, Li et al (2018) examined microbes in a constructed wetland treating polluted river water and found that denitrification rate was positively correlated with the Shannon's diversity of denitrifying bacteria measured using metabarcoding. It is, however, clear that relationships between denitrifier assemblages and denitrification rates is modulated by abiotic variables such as carbon, nitrate and ammonia availability, soil moisture, and pH, indicating the necessity to consider both biotic and abiotic factors when predicting denitrification (Shrewsbury et al., 2016; Bowen et al., 2020; Liu et al., 2020a).

Not only can different vegetation cover have different rates of nitrate leaching for an equal nitrate application rate due to differences in root structure and uptake rates (Dunbabin et al., 2003; Scherer-Lorenzen et al., 2003; Abdalla et al., 2019), but different plant residues can also alter microbial assemblages present and their ability to support denitrification (Rich et al., 2003; Truu et al., 2020; Audet et al., 2021; Martínez-Espinosa et al., 2021). For example, Rich et al (2003) compared the denitrifying communities from adjacent meadow and forest soils in Oregon, USA. They observed distinct denitrifier communities according to vegetation type and site, with a significant shift in the proportional abundances of dominant denitrifying genotypes from meadow to forest soils, and a corresponding order of magnitude higher denitrifying enzyme activity (DEA; used as a proxy for denitrification) at the meadow sites than the forest sites (Rich et al., 2003). Understanding how differences in vegetation type affect denitrifying communities, denitrification, and the resulting nitrate leaching, could help in the development of easily measured and cost-effective microbial proxies for the water quality improvement benefits yielded following land use change.

Greening Australia recently converted an old sugarcane field into approximately 4 ha of *Melaleuca* plantings as a coastal floodplain site, adjacent to Palm Creek, near Forrest Beach in north Queensland. Adjacent to the recently planted field are other distinct fields containing either weedy grass, mature *Melaleuca* trees (planted ~30 years ago), and actively farmed sugarcane. The site is also known to flood with water draining upstream sugarcane, likely resulting in nitrate deposition across all sites. This provided an ideal case study location to examine the influence of different vegetation cover on nitrate leaching. To which this study sought examine the following questions:

1. Over a 12-month period, how do the nitrate leaching rates differ between sugarcane, grass/pasture, mature *Melaleuca* Swamp, and a recently planted *Melaleuca* swamp at a flood-prone location near Ingham within Queensland's Wet Tropics? This was studied as a case study context.
2. How do nitrate leaching rates, organic matter decomposition rates and microbial assemblages differ with different nitrate application rates between sugarcane and mature *Melaleuca*? This was studied using an in-situ experiment.

3.0 Study site

This study examined nitrate leaching from four land uses within a case study farm adjacent to Palm Creek in Forrest Beach (Near Ingham), Queensland (Fig 3.1). Land uses examined included sugarcane (1.1 ha), grass (1.2 ha), mature *Melaleuca* forest (1.4 ha), and a recently planted (in 2019) *Melaleuca* forest (4.0 ha). The dominant geology at the study site consists of Quaternary coastal and estuarine sediments, with soils largely composing clay on river alluvial plains.

Palm Creek is a lowland distributary of the Herbert River, which is a 288km long river that drains a catchment area 9,842km^2. Headwaters begin in the National Parks across the Great Dividing Range within the Atherton Tablelands and travels through primarily mixed cropping and dairy farming, and then drains intensive sugarcane cropping in the lowland coastal areas near Ingham.

Figure 3.1. The locations of the four study sites used to examine nitrate leaching near Forrest beach, Queensland: Restored *Melalaeuca* (diagonal crisscross), grass (dots), mature *Melaleuca* (down sloping diagonals), and sugarcane (upward sloping diagonals).

4.0 Case study: Nitrate leaching rates from flood-prone land uses

The following methods and results seek to examine the first question, which was:

Over a 12-month period, how do the nitrate leaching rates differ between sugarcane, grass/pasture, mature *Melaleuca* Swamp, and a recently planted *Melaleuca* swamp at a flood-prone location near Ingham within Queensland's Wet Tropics?

4.1 Methods

4.1.1 Soil chemistry and biota

In characterising the soil at study sites, physicochemical soil analysis and microbial metabarcoding was carried out at the commencement the studied year (December 2021). At each of the four locations, soil samples were collecting soil from 20 randomly located soil cores to a depth of 30cm and then pooling and thoroughly mixing soil for analysis. Samples for physicochemical analysis were sent to the Environmental Analysis Laboratory at Southern Cross University, which holds both NATA and ASPAC accreditation. Laboratory analysis examined a suite of nutrients, metals, and soil characteristics (Table A1). While samples for microbial metabarcoding were sent to Metagen Australia, which included the identification of bacteria, fungi, and nematodes, using the following methods:

DNA extraction and processing

DNA was extracted from 10 g subsamples of soil using a modification of the modular universal DNA extraction protocol (Sellers et al., 2018). Briefly, this involved 10 g soil samples being mixed with sterile garnet sand and lysis buffer before being processed in a SPEX 2010 Geno Grinder homogeniser (SPEX SamplePrep, NJ) at 1700 strokes per minute for 5 minutes. After centrifugation to remove soil particles, 9 mL of the supernatant was treated with a flocculant solution designed to remove humic acid contaminants. Samples were again centrifuged, and DNA was recovered from 10 ml of the supernatant using SPRI beads (Oberacker et al., 2019).The purified DNA was then eluted in 200 µl of Tris-HCl pH 8.0 and was assessed for yield and quality using the Quantifluor dsDNA system (Promega, MI) and agarose gel electrophoresis.

Metabarcoding

Metabarcoding of eukaryotic and bacterial/archaeal communities was conducted using the primer sets NF1/18S2rB (Porazinska et al., 2009) and Pro341F/Pro805R (Takahashi et al., 2014), respectively. For analysis of soil nematode communities, the primer set Nemf/18Sr2b was used as described previously (Sikder et al., 2020).

A two-step PCR protocol was used to generate dual-indexed amplicons adapted from the Illumina protocol for 16S Metagenomic Sequencing Library Preparation. For the first PCR, each reaction contained 2 µl of template DNA, 4 µl 5X MyTaq Red PCR buffer, 0.5 µM of each gene-specific primer, and 0.2 µl MyTaq DNA polymerase in a total volume of 20 µl. PCR conditions for both the NF1/18Sr2b and 16s amplicon were 95°C for 3 min, 25 cycles of 95°C for 30 sec, 52°C for 30 sec and 72°C for 40 sec, and a final extension step at 72°C for 5 min. Amplicons from PCR1 were diluted one in ten in 10 mM Tris-HCl pH 8.0. Dual indexed PCR amplicons were produced using 2 µl diluted PCR1 amplicon as template DNA, 0.5 µM of each index primer, 5 µl 5X MyTaq Red PCR buffer, and 0.2 µl MyTaq DNA polymerase in a final volume of 25 µl. PCR conditions for the second PCR were 95°C for 3 min, 15 cycles of 95°C for 30 sec, 65°C for 30 sec, 72°C for 40 sec, followed by a final extension at 72°C for 5 min. The concentration of PCR amplicons was then measured by fluorimetry using the Quantifluor dsDNA system. Amplicons were then pooled at equimolar concentrations, purified using SPRI beads normalised to a concentration of 10 nM and sequenced by the IMB Sequencing Facility at the University of Queensland on an Illumina MiSeq (2 X 300 bp).

Sequences were demultiplexed with DeML (Renaud et al., 2015). All subsequent processing and analysis were carried out in R version 3.5.1 (R Core Team, 2021). Sequence variants and taxonomic inference was done in the DADA2 package (Callahan et al., 2016). Briefly, the read pairs were truncated to 270bp and 240bp for 18S and 16S amplicons. For the 16S amplicon reads were removed if the expected errors in the forward reads exceeded 2 and, in the reverse, if they exceeded 3. For the 18S amplicon the cut off for expected errors was 3 in the forward read and 4 in the reverse. For both amplicons chimeras were identified and removed with "consensus' method of the "removeBimeraDenovo" function. The naïve Bayesian Classifier was used to assign taxonomy to genus level for the 16S amplicon with version 128 of the Silva reference database (Quast et al 2013) and to species level with the PR2 database version 4.12 (Guillou et al., 2013) for the 18S amplicon using the "assignTaxonomy" function of DADA2.

Data analysis

The genera diversity and composition of bacterial and archaea, fungi, and nematode assemblages (microbial groups) were compared between the four sites (cane, grass, restored *Melaleuca*, and mature *Melaleuca*). Genera relative abundance was indicated by the relative number of amplicon sequence variant (ASV) reads within a sequence, and all samples were rarefied to 10,000 reads prior to analysis.

For each site, alpha diversity was indicated via the following indices: Chao1, Abundance-based Coverage Estimators (ACE), Shannon's Diversity Index, Simpson's Diversity Index, Inverse Simpson Index, and the Fisher Diversity Index. For each diversity indicator, two-way ANOVA (with a Bonferonni correction) was used to examine if each diversity indicator differed between sites.

The assemblage composition of each microbial group was compared between sites using Nonmetric Multidimensional Scaling (NMDS) ordinations with Bray-Curtis Dissimilarity. Permutational multivariate ANOVA (PERMANOVA) was used to test the statistical significance of the assemblage differences. Following Segata et al (2011), the LefSe algorithm was used to identify the taxa driving significant differences between the sites with LDA used to indicate the effect size of differentially abundant taxa (Segata et al., 2011).

The functional redundancy of various traits within the assemblages were explored by matching bacterial genera with their functional traits as documented in the Functional Annotation of Prokaryotic Taxa (FAPROTAX) database (Louca et al., 2016a, 2016b); while fungi genera were matched to traits in the FUNGuild and FungalTraits databases; and nematode genera matched to functional guilds from the Nemaplex database (Yeates et al., 1993; Bongers and Bongers, 1998; Ferris et al., 2001). The relative read abundance associated with trait was then determined for each site.

4.1.2 Leaf litter decomposition rates

At each of the 20 plots within each of the four sites, leaf litter decomposition rates were estimated over the 12-month study period by determining the loss of dry biomass from dried *Melaleuca quinquenervia* leaves buried in mesh bags (2mm aperture) at a depth of 10 cm. Biomass was measured on deployment and retrieval after drying at 70°C for 72h. Analysis of variance was used to examine the loss of biomass between the four sites, with each bag treated as an independent replicate.

4.1.3 Water levels

At each of the four study sites, HOBO™ water level loggers were deployed in 1m deep perforated sample wells to detect any incidences of rising groundwater levels or flood water inundation. The logger positions were (EPSG: 3857): sugarcane (-18.729, 146.265); mature Melaleuca (-18.729, 146.262); grass (-18.729, 146.262); and restored Melaleuca (-18.729, 146.260). Loggers measured

barometric pressure every 15 minutes and water depth calculated from the difference in barometric pressure between logger in the sample wells and a pressure logger positioned 2m aboveground (greater than the likely flooding depth). Further, at each site, an automated trail camera was positioned to capture imagery across the site every hour between (5am and 7pm) to provide a visual indication of floodwater inundation extent.

4.1.4 Nitrate leaching

Nitrate leaching rates from the topsoil into shallow groundwater were estimated at each of the four study sites using the ion-exchange resin method. This approach involved burying fine meshed bags (50 cm^2) containing 50 g of nitrate-selective ion exchange resin (Resinex NR-1) at 20 random locations at a depth of 30 cm and left in-situ for 12 months (December 2021 – December 2022). This is a high purity, premium grade, crosslinked polystyrene divinylbenzene resin that is highly selective for nitrate ions, even with high background sulphate. A nitrate-selective resin was necessary given that the sites examined are coastal with potential for interference from high sulfate soils. In the laboratory, nitrate in the resin was extracted and quantified following the methods in Technical Memo (Memo A1).

4.2 Results

4.2.1 Soil analysis

Across all four sites, soils were broadly similar (Table S1), with a clay texture, low pH (~4.5), and total carbon/nitrogen (~15), exchangeable potassium (~129 mg/kg), and high levels of phosphorus (~48 mg Colwell P/kg). The sugarcane site did, however, have greater nitrate nitrogen (3.2 mg N/kg) than the other sites (~0.9 mg N/kg). None of the sites indicated excessive heavy metals (Table S1).

4.2.2 Nitrate leaching

On average, the nitrate-N leaching was significantly ($P < 0.01$) higher at the sugarcane site (M = 17.07 kg N/ha, SD = 3.45 kg N/ha) than the grass site (M = 0.53 kg N/ha, SD = 1.53 kg N/ha), mature *Melaleuca* site (M = 0.05 kg N/ha, SD = 0.14 kg N/ha), and the restored *Melaleuca* site (M = 0.16 kg N/ha, SD = 0.33 kg N/ha).

4.2.3 Soil microbiome

Overall, the restoration site exhibited lower diversity (indicated by all three diversity indices assessed than the other three comparison sites (Table 4.1). The restoration site dominated (substantially more so than the other sites) by bacteria from the *Sphingobium* genus (Fig. 4.1), and had a negligible proportion of nitrate reducing bacteria. The mature *Melalaeuca* site and the sugarcane site had a very slight proportion of nitrate reducing bacteria (Fig. 4.2).

Table 4.1. The Chao1 Diversity, Shannon's Diversity, and Simpson's Dominance Indices of soil bacteria and archaea from the sugarcane, grass, restored *Melaleuca*, and mature *Melaleuca* study sites near Forrest Beach, Queensland. Microbial assessment carried out using metabarcoding in December 2021.

Site	Chao1	Shannon	Simpson
Restored *Melaleuca*	424	2.93	0.79
Sugarcane	682	5.99	1
Mature *Melaleuca*	709	5.67	0.99
Grass	921	6.08	0.99

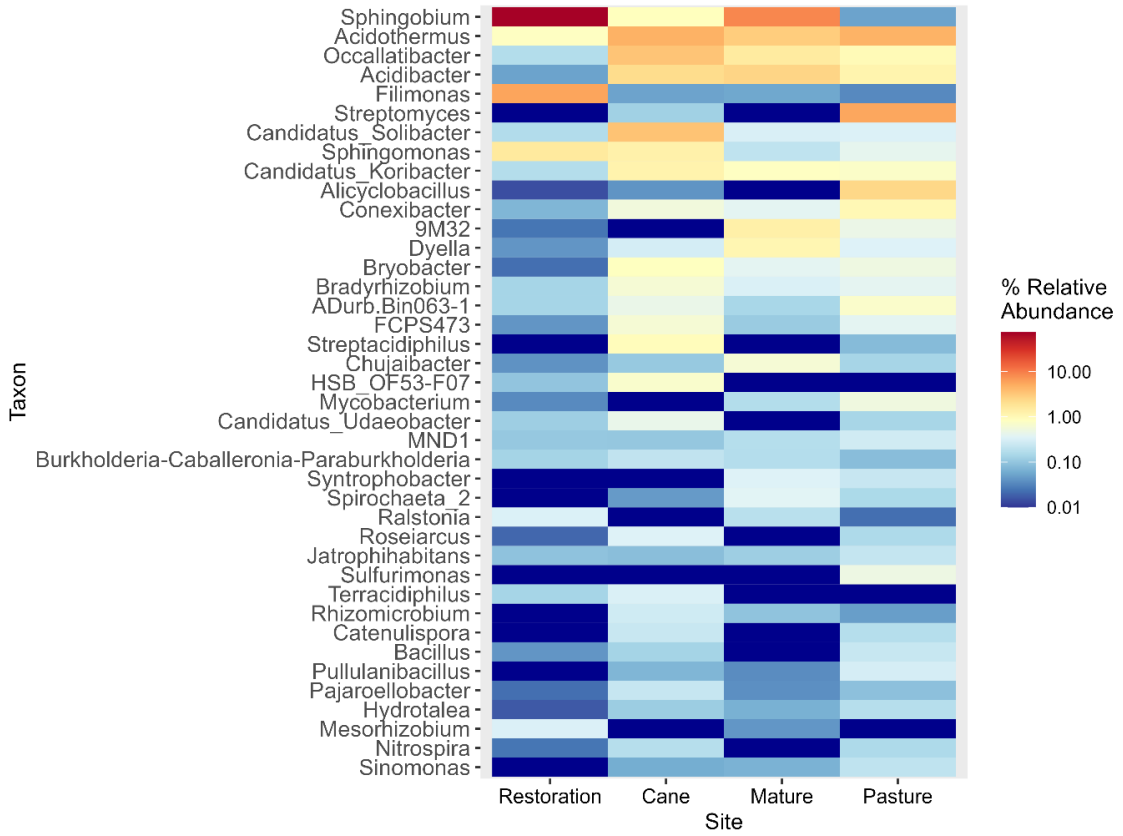


Figure 4.1. The relative read abundance of Bacteria and Archaea genera from soils at the sugarcane, grass, restored *Melaleuca*, and mature *Melaleuca* study sites near Forrest Beach, Queensland. Microbial assessment carried out using metabarcoding in December 2021.

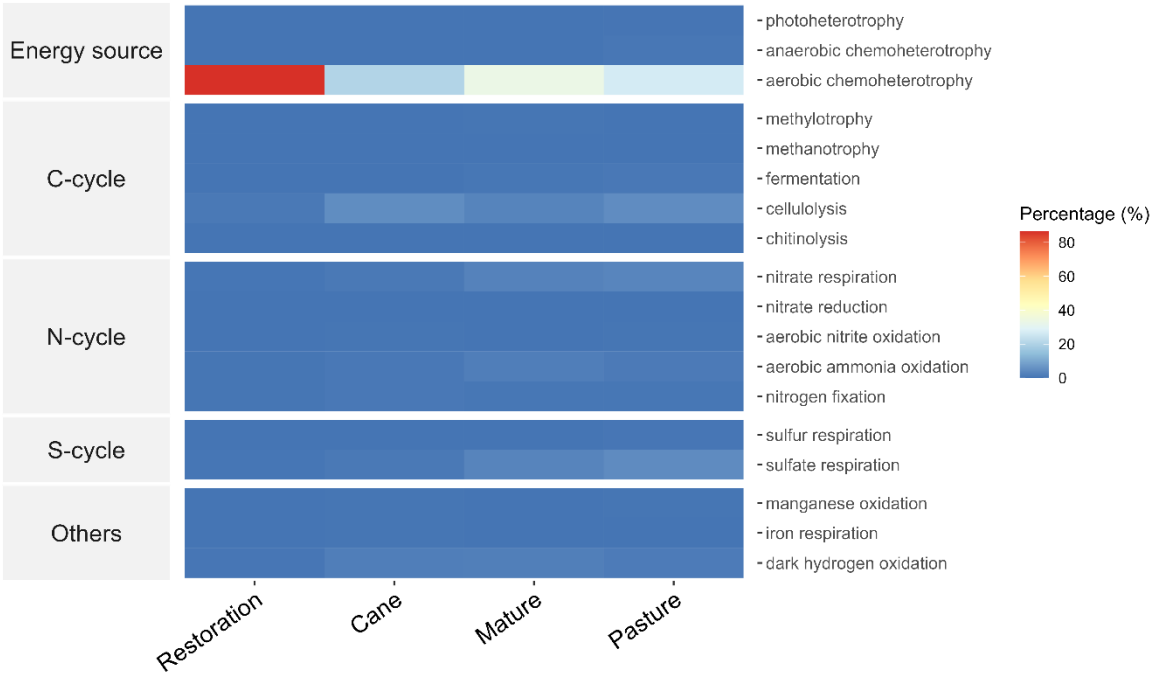


Figure 4.2. The relative read abundance of Bacteria and Archaea functional traits from soils at the sugarcane, grass, restored *Melaleuca*, and mature *Melaleuca* study sites near Forrest Beach, Queensland. Microbial assessment carried out using metabarcoding in December 2021.

4.2.4 Leaf litter decomposition rates

Across the four sites, leaf litter decomposition was significantly greater (approximately double) at the sugarcane site (M=1.47 g, P<0.01) than at the restored Melaleuca (M = 0.80 g), mature Melaleuca (M=0.94 g) and the grass (M = 0.72 g) sites, with differences between the latter three being insignificant (P>0.05, Fig. 4.3).

Figure 4.3. The leaf litter loss from 80 mesh bags of dried *Melaleuca* leaves buried at 10 cm between Dec 2021 – Dec 2022 across the sugarcane, grass, restored *Melaleuca*, and mature *Melaleuca* study sites near Forrest Beach, Queensland.

4.2.5 Water levels and rainfall

Soil water levels were consistent between all four locations, increasing following rainfall and having no base level (from groundwaters) within the depth examined (Fig. 4.4). Furthermore, all four sites exhibited ponding ~20cm deep on two occasions in late April and early May (2022), following two large rainfall events with ~200mm and ~150mm respectively (Fig. 4.4). Examination of the daily trail camera photos did not show inundation from Palm Creek throughout the study duration, with water appearing as ponding following rainfall.

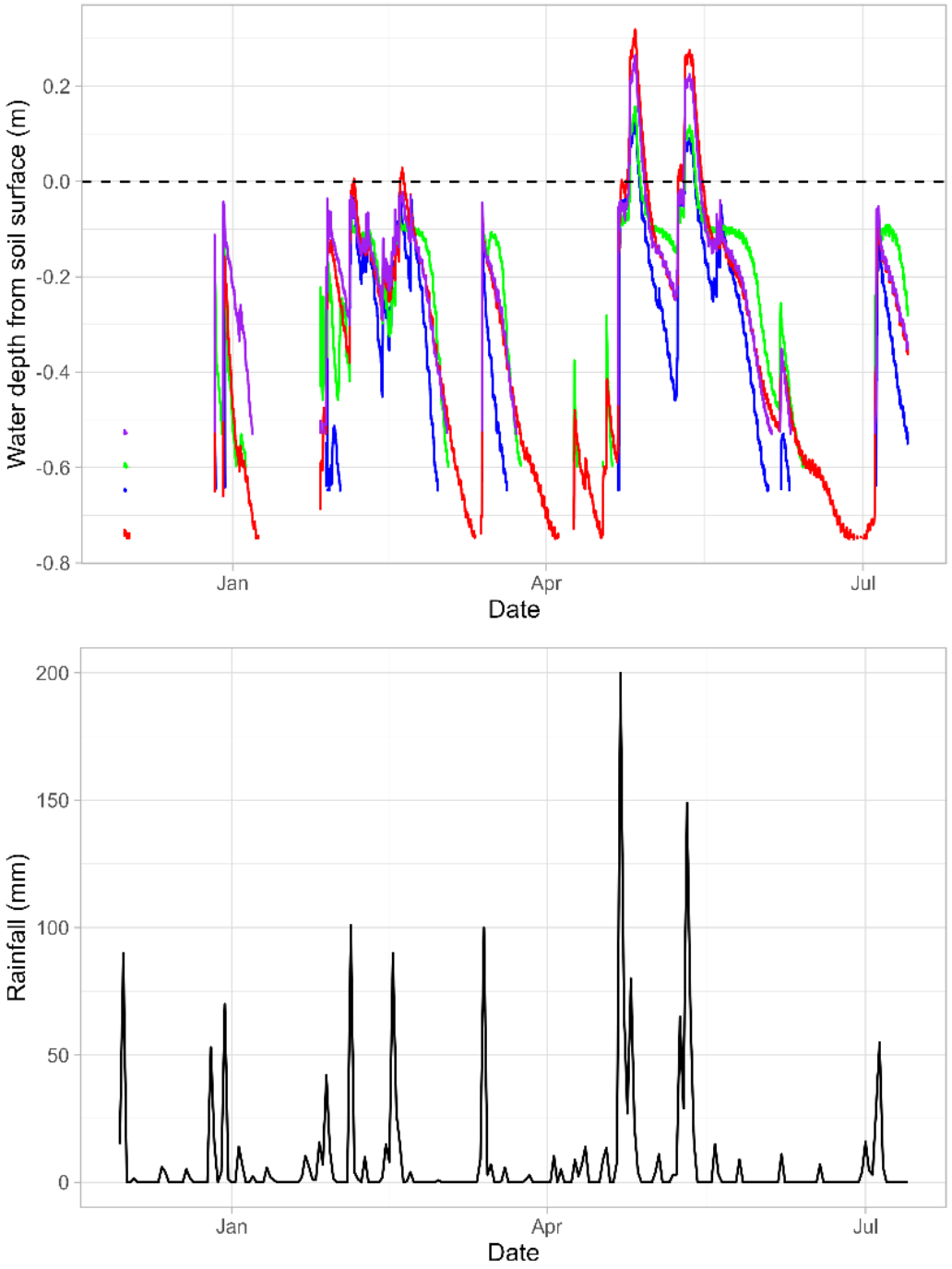


Figure 4.4. Top: The water depth (m) from the soil surface over the 2021/22 wet season the sugarcane, grass, restored *Melaleuca*, and mature *Melaleuca* study sites near Forrest Beach, Queensland. Bottom: The daily rainfall (mm) from the Ingham Composite rainfall gauge (station 032078}, sourced from the Bureau of Meteorology.

5.0 Experiment: Nitrate leaching with differing surface loading rates in sugarcane and *Melaleuca* swamp

The following methods and results seek to examine the second question, which was:

How do nitrate leaching rates, organic matter decomposition rates and microbial assemblages differ with different nitrate application rates between sugarcane and mature *Melaleuca*?

5.1 Methods

5.1.1 Soil physicochemistry

In characterising the soil within each plot, physicochemical soil analysis was carried out at the commencement and completion of the one-month experiment. Within each plot, soil samples were collected from 5 randomly located soil cores to a depth of 10 cm, pooled, thoroughly mixed, and sent for analysis at the Environmental Analysis Laboratory at Southern Cross University, which holds both NATA and ASPAC accreditation. Laboratory analysis examined the following characteristics: pH and electrical conductivity (1:5 water); exchangeable cations (sodium, potassium, calcium, magnesium, hydrogen, and aluminium); cation exchange capacity; Colwell phosphorus; total carbon (TC), Total Nitrogen (TN), organic matter; and a description of the basic colour and texture.

5.1.2 Leaching experiment

In this experiment, nitrate leaching from topsoil was examined one month after the application of nitrate, at five different nitrogen loading rates, across soil plots (2m x 2m) within the sugarcane and mature restored *Melaleuca* swamp sites described above. The experiment was conducted for six weeks between the 11th of November 2022 and the 23rd of December 2022 to capture the onset of monsoonal rains when accumulated nutrients are most mobilised. Nitrogen was applied as potassium nitrate (KNO_3) dissolved in 2L of water, evenly spread over 2m-by-2m soil patches. Application rates were 0 kg N/ha (control), 25 kg N/ha, 50 kg N ha, 75 kg N/ha, and 100 kg N/ha, with plots replicated in three random locations across the sugarcane patch and three random locations across the *Melaleuca* patch, yielding a total of 30 plots over the entire experiment. Nitrate leaching from the topsoil (at 30 cm depth) into shallow groundwater over the month study period was estimated using the ion-exchange resin method (explained in section 4.1.4), with one resin bag buried in the centre of each plot prior to the nitrogen application.

5.1.3 Decomposition rates

Wood decomposition rates were compared between plots by burying wooden stirring sticks (~15cm x 1.5 cm x 0.2 cm) under 10 cm of soil at three locations per plot, and quantifying decomposition by subtracting the dried mass after the one-month experiment from that measured prior to the experiment. Sticks were dried before and after the experiment for three days at 70°C and weighed to the nearest milligram. Two-way analysis of variance (ANOVA) was used to examine the loss of biomass between the sites and treatments, with each plot treated as an independent replicate.

5.1.4 Microbial assemblages

Microbial metabarcoding was used to examine the soil microbial assemblages within each plot at the commencement and completion of the one-month experiment. Within each plot, soil samples were collected from 5 randomly located soil cores to a depth of 10 cm, pooled, thoroughly mixed, and sent for metabarcoding at Metagen Australia. Metabarcoding included the identification of bacteria, fungi and nematodes following the amplification of 16S rRNA, 18S rRNA and a nematode-specific markers. Abundance was indicated by the number of amplicon sequence variant (ASV) reads. The

metabarcoding methods and data analysis were the same as those outlined in section 4.1.1. Shannon's Diversity Index was used to examine the total diversity, and the diversity of nitrate reducing taxa.

5.2 Results

5.2.1 Soil characteristics

All soil samples had similar texture, nitrate-N, and total C:N. Relative to the *Melaleuca* plots, the cane sites had slightly higher pH in water but slightly lower pH in $CaCl_2$, lower electrical conductivity, lower ammonium-N, lower cation exchange capacity, and lower organic carbon (Figure 5.1).

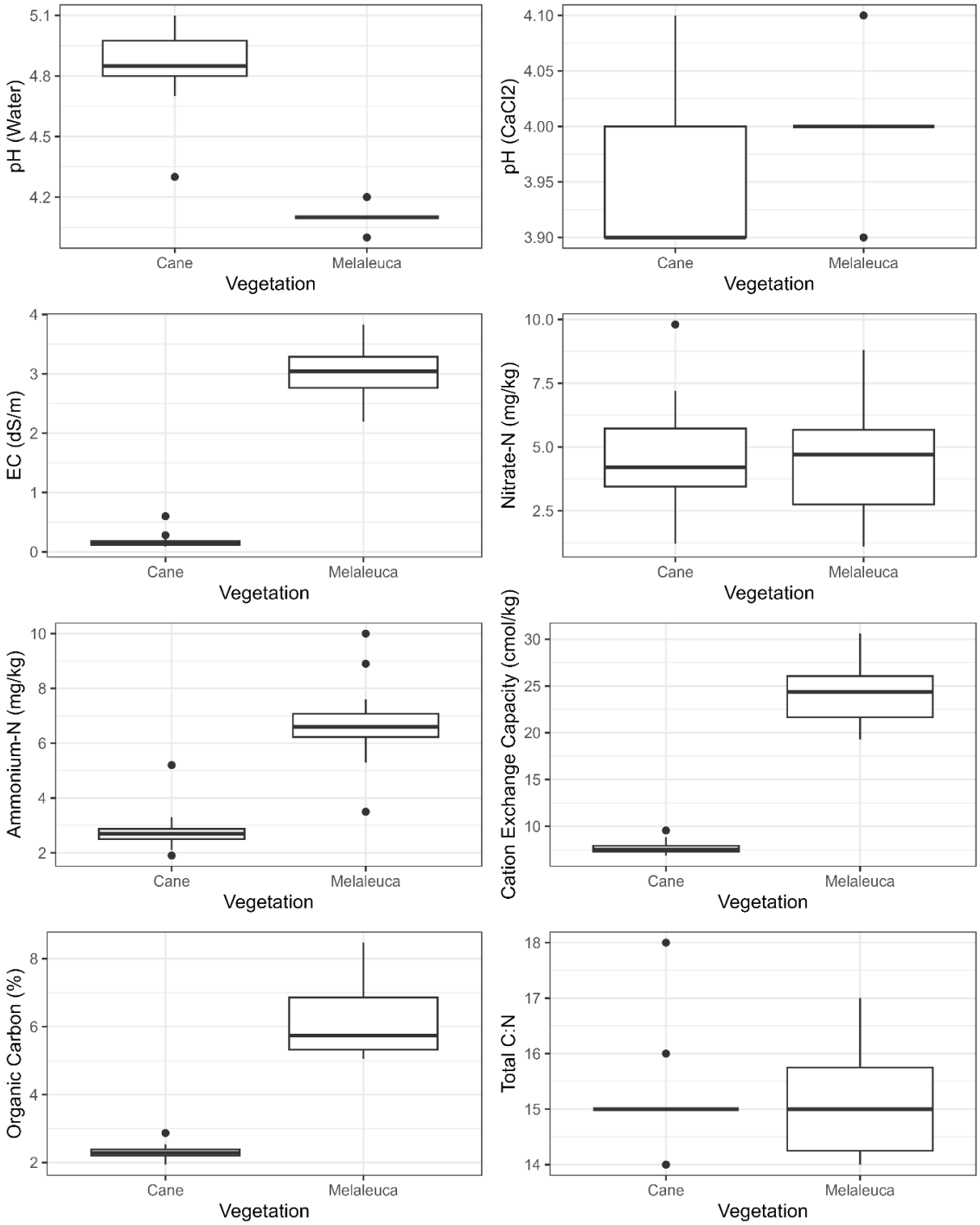


Figure 5.1. Boxplot summary statistics for eight soil characteristics across the individual experimental plots at the sugarcane site and the *Melaleuca* site.

5.2.2 Nitrate leaching

Overall, increased nitrate loading resulted in greater nitrate leaching at the cane site but not the mature *Melaleuca* site, though there was considerable scatter that was unexplained by the prior C:N ratio (Fig. 5.2; Table 5.1).

Table 5.1. The statistics for a regression fitted to estimate nitrate (N) leaching from the applied N loading, vegetation type, initial C:N ratio, and an interaction between N loading and vegetation.

Parameter	Estimate	Standard error	t value	P-value
Intercept	212.77	126.45	1.68	0.11
N loading	1.46	0.29	5.12	<0.001
Vegetation	32.49	28.64	1.13	0.27
C:N Ratio	-14.73	8.55	-1.72	0.10
N loading X vegetation	-1.58	0.50	-3.15	0.006
Adjusted R^2			0.59	
F-statistic$_{4,17}$			8.56	
p-value			<0.001	

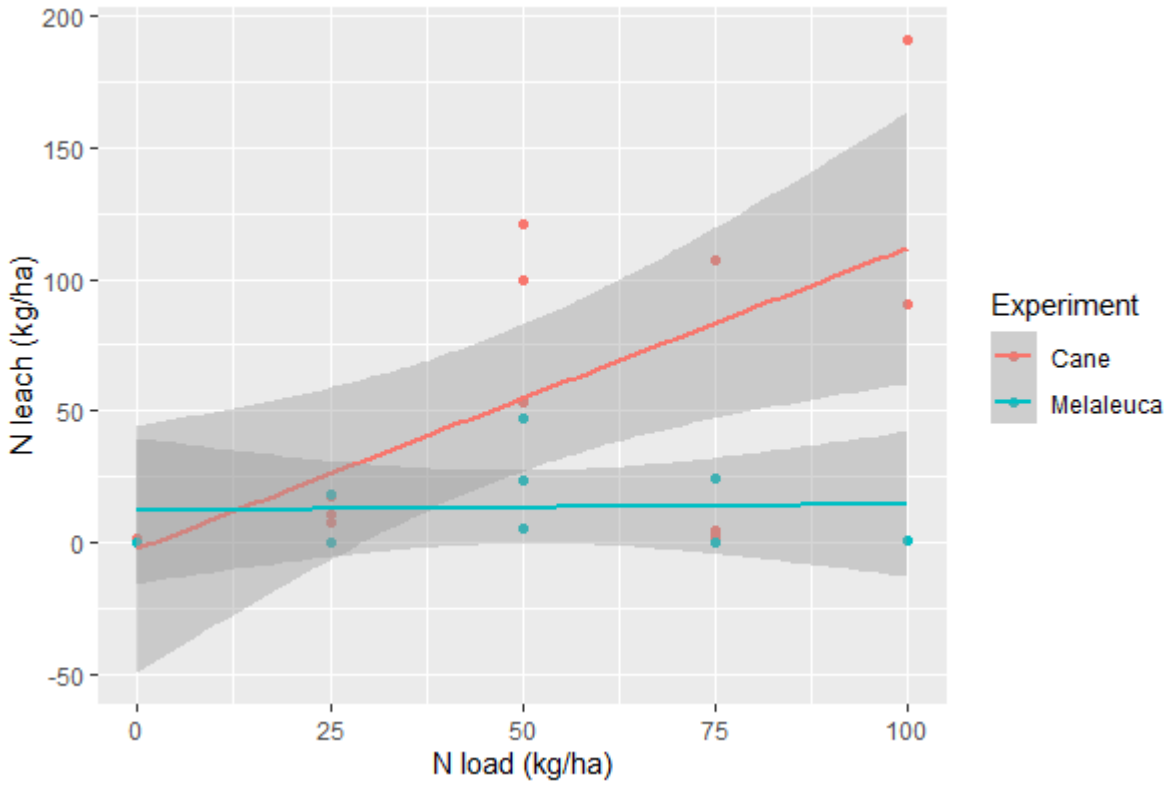


Figure 5.2. The regressions between N leaching for a given applied N load at experimental plots across a sugarcane site and an adjacent *Melaleuca* site near Forrest Beach, Queensland.

5.2.3 Wood decomposition

Regardless of nitrate loading, wood decomposition rates were significantly greater in the sugarcane site than the mature *Melaleuca* site (Fig. 5.3; Table 5.2).

Table 5.2. The statistics for a regression fitted to estimate wood decomposition (%) from the applied N loading, vegetation type, initial C:N ratio, and an interaction between N loading and vegetation.

Parameter	Estimate	Standard error	t value	P-value
Intercept	10.50	6.33	1.66	0.012
N load	0.04	0.014	2.62	0.018
Vegetation	-4.21	1.43	-2.94	0.009
C:N Ratio	0.22	0.43	0.51	0.619
N load X vegetation	-0.02	0.026	-0.71	0.49
Adjusted R^2			0.71	
F-statistic$_{4,17}$			13.62	
p-value			<0.001	

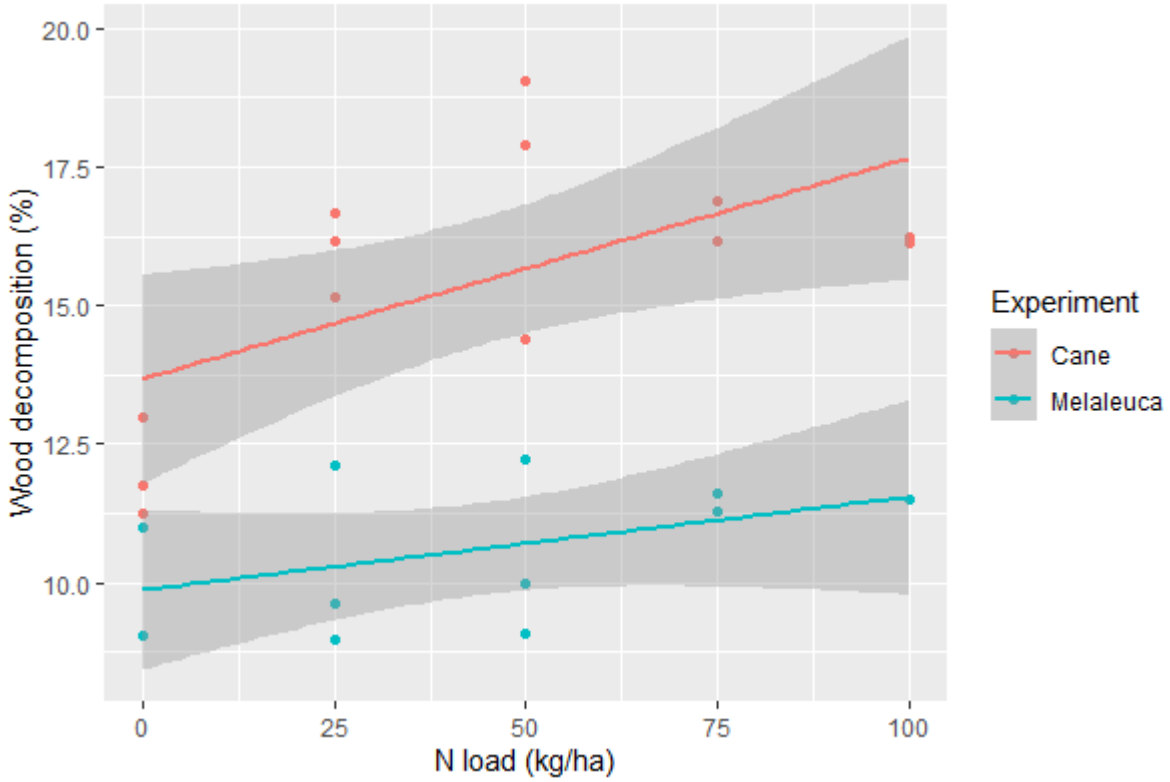


Figure 5.3. The regressions between wood decomposition (%) for a given applied N load at experimental plots across a sugarcane site and an adjacent *Melaleuca* site near Forrest Beach, Queensland.

5.2.4 Microbial assemblages

Sampling time and individual plots but not the applied nitrate load nor the vegetation affected microbial alpha diversity as indicted by the Shannon's Diversity Index (Table 5.3, Figure 5.4).

Table 5.3. The statistics for a mixed effects regression fitted to estimate the Shannon's Diversity Indices of all bacteria and archaea assemblages from the applied N loading and the vegetation type (as fixed effects), and the individual plot and sampling date as random effects (RE).

Parameter	Estimate	Standard error	DF	t value	P-value
Intercept	5.49	0.51	2.02	10.86	0.008
N loading	-0.0003	0.001	54.12	-0.242	0.81
Vegetation	0.07	0.08	27.09	0.89	0.38
Plot (RE)	-	-	-	-	0.71
Date (RE)	-	-	-	-	<0.001

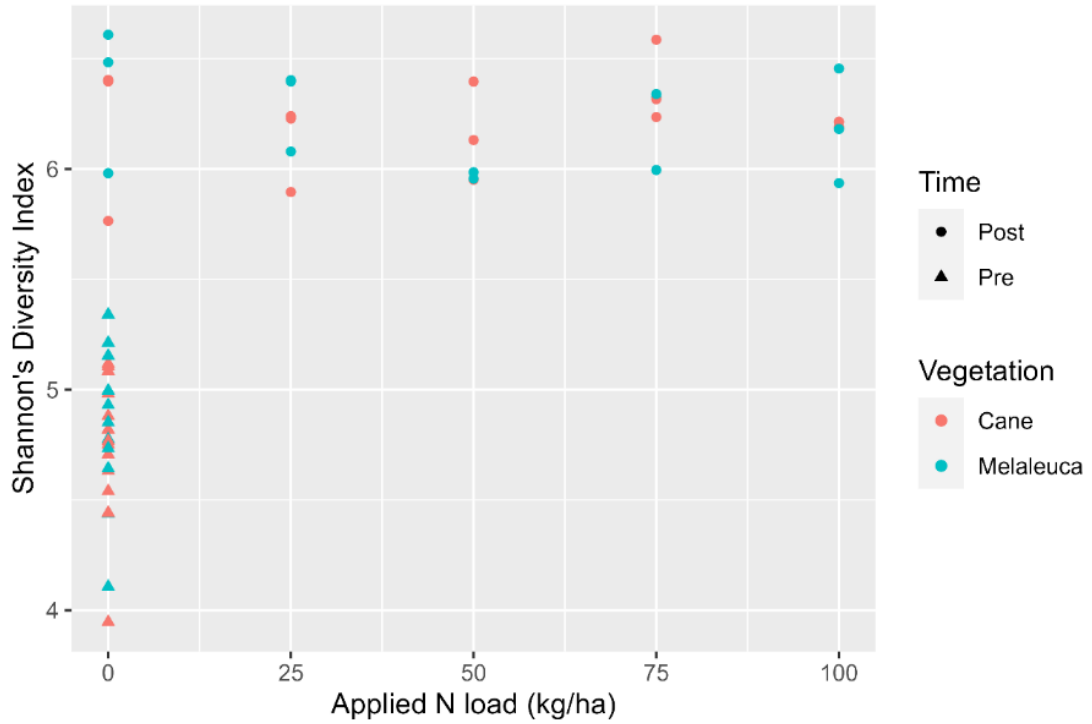


Figure 5.4. The Shannon's diversity of bacterial and archaea assemblages (using 16S metabarcoding) before and after an a given experimental nitrate application load at experimental plots across a sugarcane site and an adjacent *Melaleuca* site near Forrest Beach, Queensland.

There was a significant positive correlation between the Shannon's Diversity of the known nitrate reducing taxa with applied nitrate loading, and this was not affected by vegetation type as a fixed effect, nor was plot or date influential as random effects (Table 5.4, Figure 5.5). Nitrate reducing taxa represented a very small proportion of all taxa within all plots where observed, and were significantly different between sample dates but did not differ with applied nitrogen loading, vegetation type or plot (Table 5.5, Figure 5.6).

Table 5.4. The statistics for a linear mixed-effect model (maximum likelihood) fitted to estimate the Shannon's Diversity of nitrate reducers from the applied N loading (N load) and vegetation type (Cane vs *Melaleuca*) as fixed effects, and the experimental plot and sample date as random effects. Data collected from a trial location near Forrest Beach, Queensland, and microbial abundance assessed using the relative read abundance from 16S metabarcoding, rarefied to 10,000 reads.

Parameter	Estimate	Standard error	t value	P-value
Intercept	0.92	0.13	7.27	<0.01
N load	0.0067	0.002	3.02	<0.01
Vegetation	0.11	0.14	0.76	0.46
Plot (RE)	-	-	-	-
Date (RE)	-	-	-	-

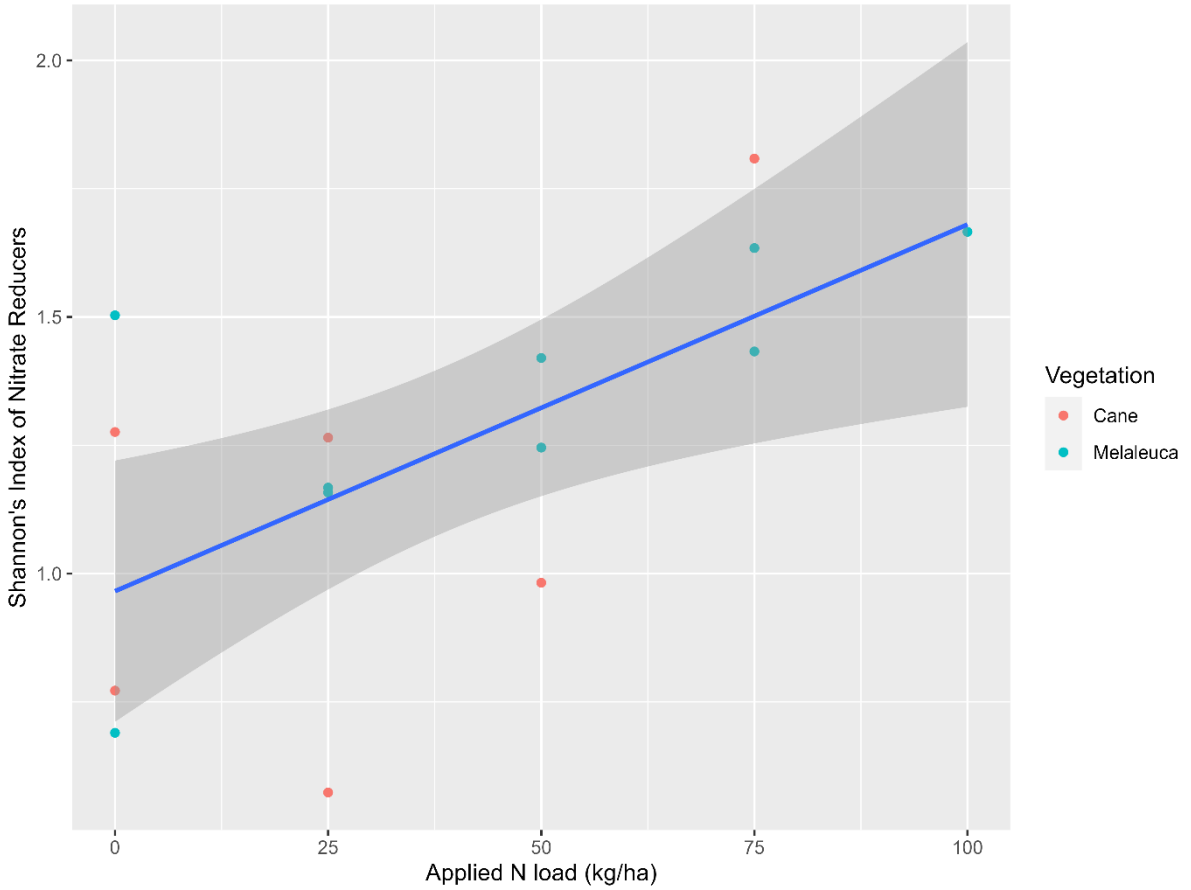


Figure 5.5. The (non-zero) Shannon's diversity of nitrate reducers (using 16S metabarcoding, rarefied to 10,000 reads) following a given experimental nitrate application load at experimental plots across a sugarcane site and an adjacent *Melaleuca* site near Forrest Beach, Queensland.

Table 5.5. The statistics for a linear mixed-effect model (maximum likelihood) fitted to estimate the relative abundance of nitrate reducers from the applied N loading (N load) and vegetation type (Cane vs *Melaleuca*) as fixed effects, and the experimental plot and sample date as random effects. Data collected from a trial location near Forrest Beach, Queensland, and microbial abundance assessed using the relative read abundance from 16S metabarcoding, rarefied to 10,000 reads.

Parameter	Estimate	Standard error	t value	P-value
Intercept	0.25	0.13	1.87	0.19
N load	$9.42E^{-4}$	$8.05E^{-4}$	1.17	0.25
Vegetation	$5.95E^{-3}$	$4.56E^{-2}$	0.13	0.90
Plot (RE)	-	-	-	0.24
Date (RE)	-	-	-	<0.001

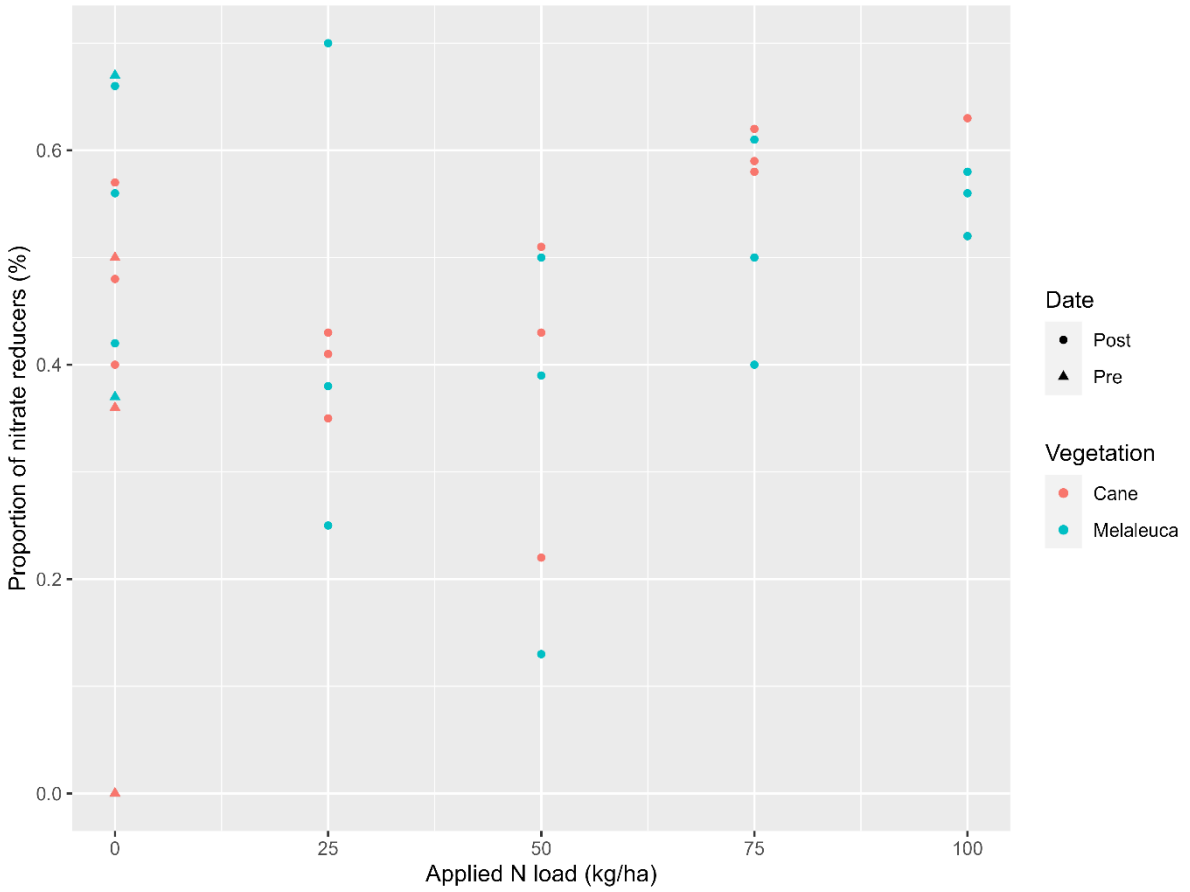


Figure 5.6. The relative read abundance of nitrate reducing taxa (using 16S metabarcoding, rarefied to 10,000 reads) before and after a given experimental nitrate application load at experimental plots across a sugarcane site and an adjacent *Melaleuca* site near Forrest Beach, Queensland. All pre-application samples have an applied N load of zero.

The soil microbial assemblages were distinctly different between the sugarcane and mature *Melalaeuca* sites, over the duration of the experiment the assemblages did become more similar (but still distinct) though this change was independent of the nitrate load (Table 5.6, Figure 5.7).

Table 5.6. The statistics for a PERMANOVA using Bray-Curtis Dissimilarity of bacterial and archaea assemblages (using 16S metabarcoding, rarefied to 10,000 reads) before and after an a given experimental nitrate application load at experimental plots across a sugarcane site and an adjacent *Melaleuca* site near Forrest Beach, Queensland.

Factor	DF	SumOfSqs	R^2	F-stat	P-value
Vegetation	1	3.207	0.152	19.965	0.001
Date	1	6.080	0.287	37.843	0.001
Nitrate load	1	0.208	0.010	1.297	0.201
Plot	1	0.340	0.016	2.116	0.041
Vegetation X Date	1	3.226	0.153	20.081	0.001
Vegetation X Nitrate load	1	0.178	0.008	1.109	0.312
Date X Nitrate load	1	0.191	0.009	1.189	0.280
Vegetation X Date X Nitrate load	1	0.174	0.008	1.082	0.350
Residual	47	7.551	0.357		
Total	55	21.156	1.000		

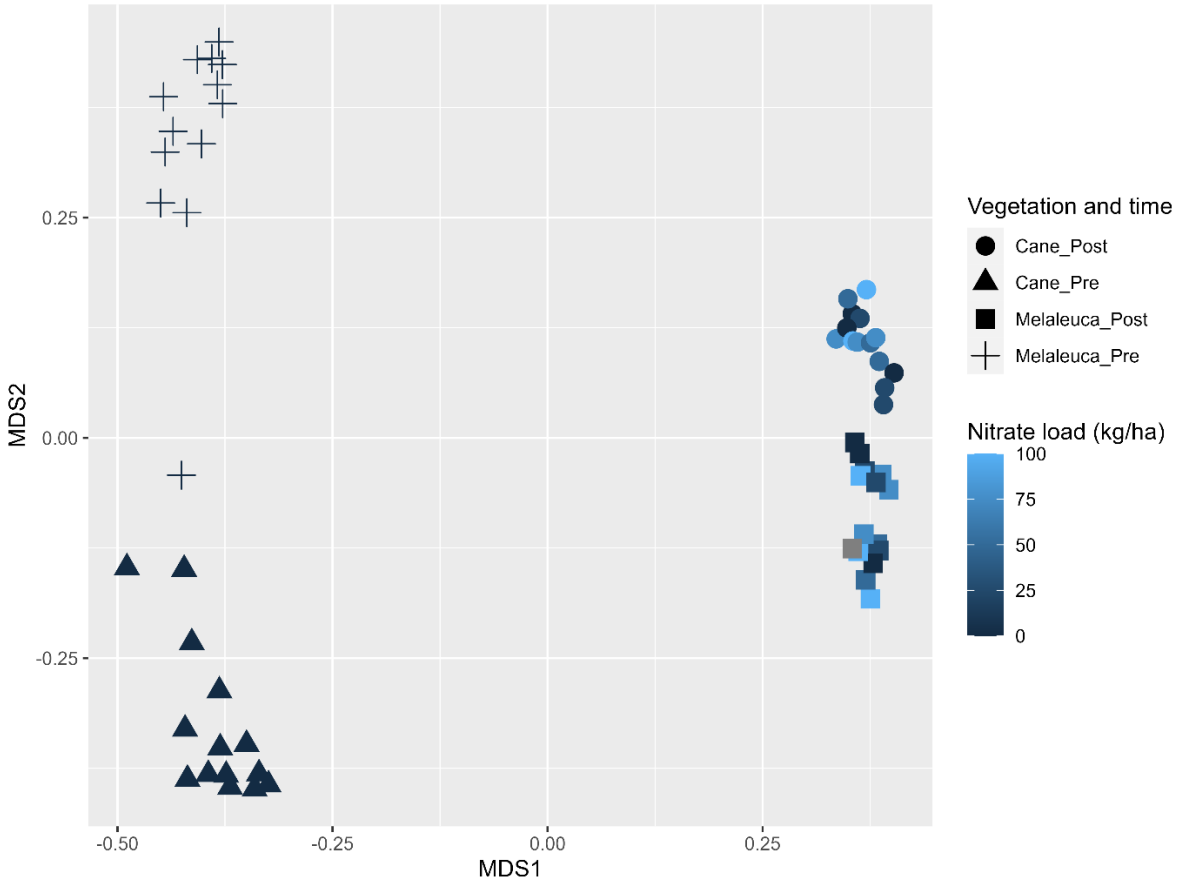


Figure 5.7. An NMDS using Bray-Curtis Dissimilarity of bacterial and archaea assemblages (using 16S metabarcoding, rarefied to 10,000 reads) before and after an a given experimental nitrate application load at experimental plots across a sugarcane site and an adjacent *Melaleuca* site near Forrest Beach, Queensland.

The differences between the sampling dates at the *Melaleuca* site were a considerable reduction in the relative read abundances of the genera *Acidibacter*, 9M32, *Acidiphilium*, *Alkanibacter*, *Occallatibacter*, and *Ignavibacterium*, while there were increases in the genera *Alicyclobacillus*, 1921-2, *Piscinibacter*, and RB41. While the difference between sampling dates at the cane site were a considerable reduction in the relative read abundances of the genera *Acidibacter*, *Occallatibacter*, and HSB_OF53-F07, while there were increases in the genera *Alicyclobacillus,* RB41, Spirochaeta, and *Desulfuromonas* (Figure 5.8).

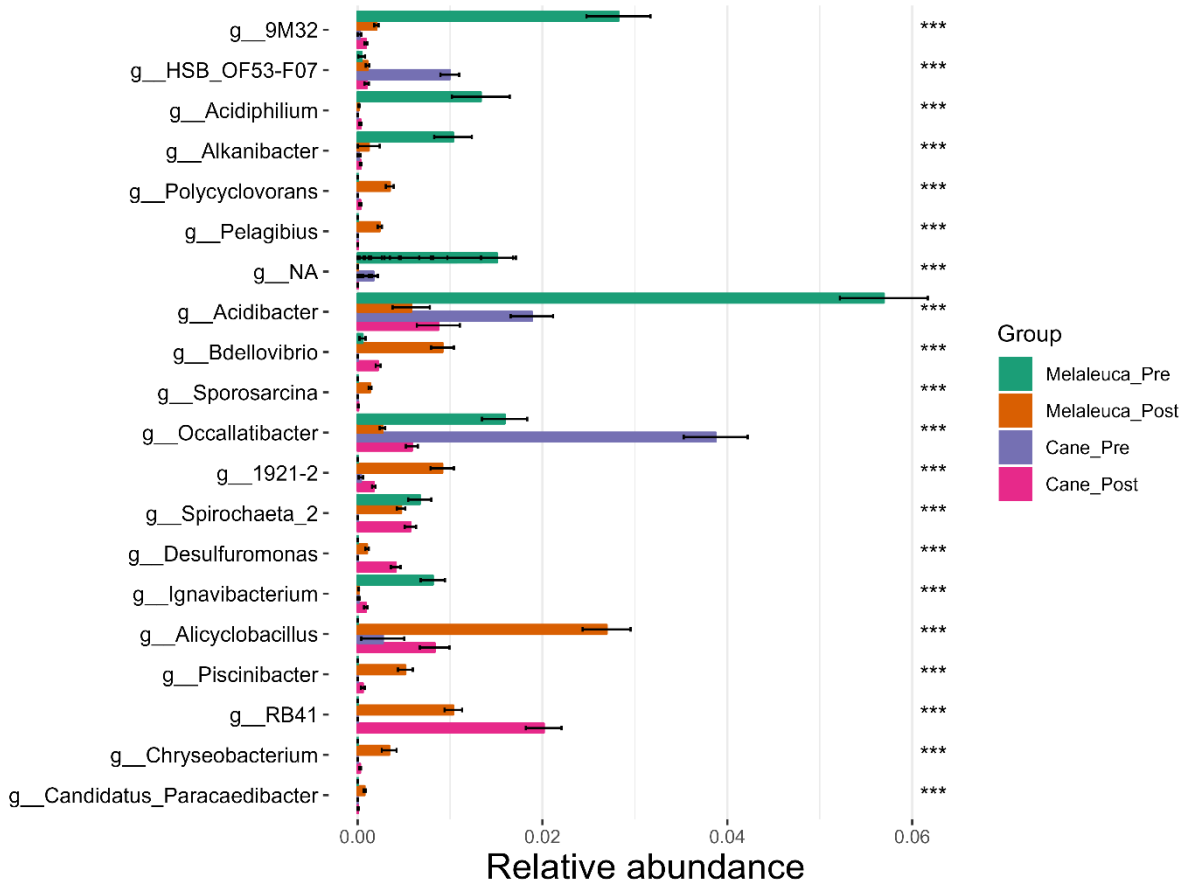


Figure 5.8. The relative read abundance of soil bacteria and archaea (using 16S metabarcoding, rarefied to 10,000 reads) of individual genera identified as most influential in the dissimilarity before and after an a given experimental nitrate application load at experimental plots across a sugarcane site and an adjacent *Melaleuca* site near Forrest Beach, Queensland. Taxa were identified as those with with the largest LDA effect size calculated by the LefSe algorithm by Segata et al (2011). The asterix indicates the P-value (false discovery rate adjusted) from Kruskal-Wallis Rank Sum Tests comparing relative abundance between groups. All taxa depicted showed '***' indicating a P-value < 0.001.

6.0 Discussion

6.1 Long term trial

Over the year (Dec 2021-22), the nitrate leaching rates were substantially higher at the sugarcane site than the other three sites, which were similarly very low. the measured sugarcane leaching rates are typical of sugarcane elsewhere within the GBR's Wet Tropics, with Stewart et al (2006) estimating a likely range of 14–24 kg/ha per season, and McCloskey et al (2021) estimating an average annual flux of 12.8 kg/ha. The differences observed are unlikely driven by sub-surface or floodwater nitrate delivery, and likely attributed to the sugarcane site having greater rates of fertilisation and mineralisation encouraged by soil tillage aeration.

As the soil water peaks were sharp and short, coinciding only with high rainfall events over the wet season, the influence of shallow aquifer upwelling is unlikely. Furthermore, in the time-lapse photography observations, water appeared to be ponding rather than from flood waters sourced from the adjacent Palm Creek or cane drains. These suggest that nitrate across the sites was unlikely sourced from sub-surface or flood waters.

The sugarcane site also had twice the leaf litter decomposition of the other three sites, indicating greater microbial activity and likely greater mineralization of nitrogen from soil organic matter into inorganic forms, such as nitrate, which is prone to leaching. Differences in the decomposition rate is unlikely driven by the carbon:nitrogen (C:N) ratio as the sugarcane site had a very similar ratio to both the recently restored and the matured *Melaleuca* sites. Rather it is likely driven by the sugarcane site having greater soil aeration from tilling and greater nutrient application.

All the sites also had very small portions of denitrifying bacteria and nitrogen fixing bacteria, suggesting that both the removal and addition of nitrate by soil microbes was unlikely influential. Instead, the restoration site was dominated (substantially more so than the other sites) by bacteria from the *Sphingobium* genus, which metabolizes and degrades is 3- or 4-ring polycyclic aromatic hydrocarbons (PAHs). PAHs can arise naturally following the burning of organic matter or artificially following burning or the application of pesticides and herbicides. This is likely explained by burning that occurred prior to planting by the landholder; while herbicides were used in weed control they were used sparingly and localized.

6.2 Experimental trials

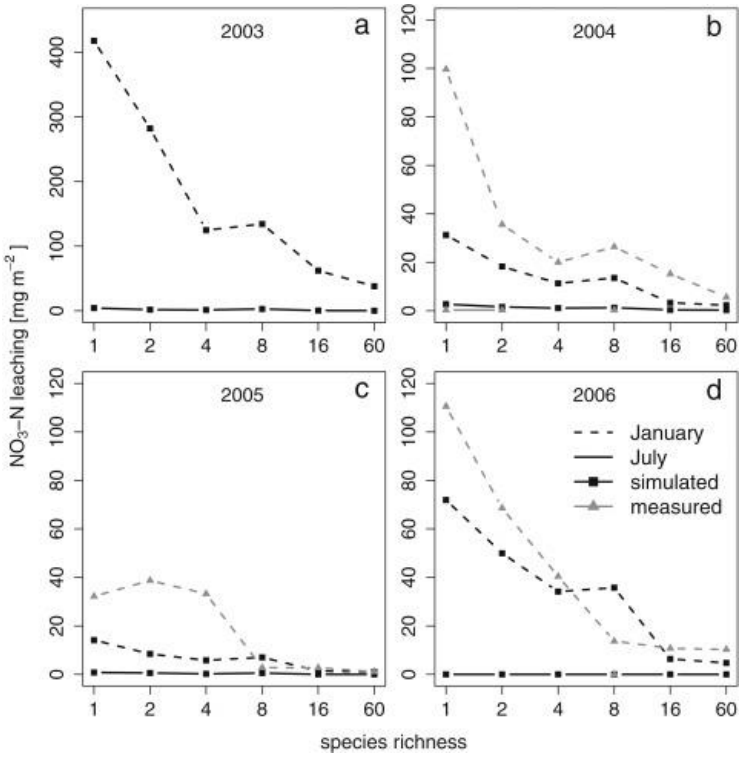
While the long-term trial did not flood, *Melaleuca* restorations may occur in locations that do flood with nitrate-laden waters, typically observed at the onset of the wet season. The nitrate leaching and soil microbial assemblages were examined using experimental plots at the mature *Melaleuca* and sugarcane cropping sites following the application of different rates of nitrate during the onset of the wet season. Overall, increased rates of applied nitrate resulted in greater nitrate leaching at the sugarcane site but not the mature *Melaleuca* site, though there was considerable scatter that was unexplained by the prior C:N ratio.

When examining individual plots, some plots showed leaching greater than rate of nitrate applied, while others showed reduced rates. This likely reflects variability in the preferential flow pathways within soils as water does not move through soil as a homogenous blanket. Rather water (carrying nitrates) will aggregate along preferential flow pathways with some resin packs likely intercepting these pathways more so than others. Additionally, the greater decomposition rates at the sugarcane site, also likely driven by the aeration from tillage and the fertilisation, would result in more mineralisation of organic nitrogen into the more easily leached inorganic nitrate form.

It is plausible that the *Melaleuca* site showed substantially less leaching than the sugarcane site as the *Melaleuca* plots typically had ~2-3x the organic carbon of the sugarcane plots, thereby enabling greater growth in microbial biomass which immobilises nitrate into organic forms. High rates of nitrate reduction were unlikely at either site as nitrate reducing bacteria represented a very small proportion of all microbes within all plots, regardless of applied nitrate load or vegetation. A very low abundance of nitrate reducing bacteria may have been driven by a lack of both abundant nitrate and anoxic conditions – both required to support nitrate reducing bacteria populations. That said, the Shannon's Diversity of the known nitrate reducing taxa increased significantly with an increased applied nitrate load, regardless of vegetation type. In constructed water treatment wetland, Li et al (2018) observed a positive relationship between the Shannon's Diversity of nitrate reducing bacteria and denitrification, while Cavigelli and Robertson (2001) observed a correlation between denitrifier diversity and nitrous oxide production, which is produced during incomplete denitrification. It remains unclear whether these relationships apply broadly or consistently or the extent to which other factors, such as acidity, mediate the relationship (Hagh-Doust et al., 2023). If the relationship is broadly applicable, then the continued and consistent application of nitrate beyond the length of this experiment may continue to increase nitrate reducer diversity that yield observable impacts on nitrate leaching (Liu et al., 2020b). Furthermore, this study essentially examined the microbial responses to nitrate addition in two monocultures (*Melaleuca* and sugarcane), and this may explain why vegetation was not a significant predictor of nitrate reducer diversity. If sporadic pulses of nitrate delivery and anoxic conditions, as observed in floods, are insufficient to maintain the diversity of nitrate reducing bacteria and the capacity for the rapid onset of high denitrification rates then other approaches to cultivating the denitrifying community should be explored (Song et al., 2010; Bender et al., 2016; Ye et al., 2017). This could include increasing the functional diversity of plants (e.g., with intercropping, multispecies cover crops, or diverse revegetation plantings) (Sutton-Grier et al., 2011; Pajares and Bohannan, 2016; Choudhury et al., 2022), and altering agriculture practices, such as reducing tillage (Smith et al., 2010; Wang and Zou, 2020; Bösch et al., 2022), changing irrigation (Korbel et al., 2022), and switching to organic fertiliser (Kramer et al., 2006).

6.3 Recommendations

1. Use a combination of satellite imagery and catchment modelling to identify locations that flood regularly with nutrient enriched waters and have capacity for improved management. As riparian zones not only intercept overland runoff, but they are more frequently inundated by river water than further removed floodplains, they likely have greater ability to remove nutrients and deliver improved water quality.
2. Carry out trials to examine multi-species plant combinations that are most effective at reducing nutrient loss. Several studies have demonstrated that nitrate leaching from the root zone into groundwaters can be substantially reduced with increased plant diversity (Scherer-Lorenzen et al., 2003; Bingham and Biondini, 2011; Leimer et al., 2015, 2016). Greater plant diversity likely drives more exhaustive resource use because of higher diversity in resource acquisition strategies e.g., more variation in rooting depth or seasonal activity. Leimer et al (2015) experimentally examined nitrate leaching between 2003 and 2006 in a grassland plant diversity experiment in Jena, Germany which consisted of 82 plots with 1–60 plant species and 1–4 plant functional groups (legumes, grasses, non-leguminous tall herbs, and non-leguminous small herbs). The results are presented in the figure reproduced below, which indicate there are diminishing returns with increasing species richness, with around 4-8 species being the point of inflection.



3. In addition to examining different multi-species plant combinations, also repeat trials under soil types to examine the consistency in nitrate removal with different soils. Simultaneously, assess whether microbial traits (such as nitrate reducing metabolism) is a robust indicator of nitrate removal – which could potentially lead to a more cost-effective and pragmatic way to indicate the nitrate removal efficacy of improved land management.

6.4 Conclusions

7. For the calendar year between December 2021 and 2022, the nitrate leaching from the examined low-lying sugarcane site was approximately 17 kg N/ha, which was substantially greater than the grass site, mature *Melaleuca* site, and the recently restored *Melaleuca* site, of which all three leached <1 kg N/ha. This indicates that retiring land from low-lying sugarcane can essentially eliminate nitrate leaching altogether.
8. All four adjacent land uses examined had very low proportions of nitrate reducing bacteria, likely arising from a lack a persistent nitrate (at high levels) and anoxic conditions. As a result, short term deliveries of nitrate are unlikely to be reduced as the populations required to reduce nitrate are not maintained in between events.
9. Nitrate applied experimentally to soils beneath sugarcane and mature *Melaleuca* leached considerably in the sugarcane but not in the mature *Melaleuca*. It is hypothesized that the high soil carbon in the mature *Melaleuca* site likely enabled greater microbial growth and immobilization of inorganic nitrate, thus reducing leaching.
10. It is recommended that future studies scope locations where planting can occur to ensure vegetation is regularly inundated by nitrate-laden waters, and the efficacy of multispecies plantings to bolster denitrification capacity is explored.

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Appendices

Table A1. Baseline (Dec 2021) soil chemistry assessments from the four sites prior to a long-term assessment of nitrate leaching.

Parameter	Method reference	Sugarcane	Recently planted Melaleuca	Mature Melaleuca	Grass
Soluble Calcium (mg/kg)	**Inhouse S10 - Morgan 1	145	279	243	214
Soluble Magnesium (mg/kg)		130	290	443	357
Soluble Potassium (mg/kg)		31	57	42	51
Soluble Phosphorus (mg/kg)		<1	<1	<1	<1
Phosphorus (mg/kg P)	**Rayment & Lyons 2011 - 9E2 (Bray 1)	21	12	21	9.4
	**Rayment & Lyons 2011 - 9B2 (Colwell)	63	43	50	37
	**Inhouse S3A (Bray 2)	54	26	52	23
Nitrate Nitrogen (mg/kg N)	**Inhouse S37 (KCl)	3.2	1.3	0.80	0.60
Ammonium Nitrogen (mg/kg N)		13	26	22	8.1
Sulfur (mg/kg S)		63	181	798	303
pH	Rayment & Lyons 2011 - 4A1 (1:5 Water)	4.62	4.82	4.15	4.27
Electrical Conductivity (dS/m)	Rayment & Lyons 2011 - 3A1 (1:5 Water)	0.207	0.524	2.202	1.783
Estimated Organic Matter (% OM)	**Calculation: Total Carbon x 1.75	5.4	3.9	5.9	3.5
Exchangeable Calcium (cmol$_+$/kg)	Rayment & Lyons 2011 - 15D3 (Ammonium Acetate)	1.2	2.6	2.0	1.8
(kg/ha)		543	1,149	890	815
(mg/kg)		242	513	397	364
Exchangeable Magnesium (cmol$_+$/kg)		2.0	4.2	5.6	4.6
(kg/ha)		532	1,139	1,522	1,243
(mg/kg)		237	508	679	555
Exchangeable Potassium (cmol$_+$/kg)		0.26	0.37	0.32	0.36
(kg/ha)		225	328	284	319
(mg/kg)		100	146	127	142
Exchangeable Sodium (cmol$_+$/kg)		1.0	2.3	7.7	6.3
(kg/ha)		540	1,203	3,981	3,262
(mg/kg)		241	537	1,777	1,456
Exchangeable Aluminium (cmol$_+$/kg)	**Inhouse S37 (KCl)	3.3	0.78	2.9	1.3
(kg/ha)		663	158	580	264
(mg/kg)		296	70	259	118
Exchangeable Hydrogen (cmol$_+$/kg)	**Rayment & Lyons 2011 - 15G1 (Acidity Titration)	8.0	3.3	8.9	5.5
(kg/ha)		180	74	200	123
(mg/kg)		80	33	89	55
Effective Cation Exchange Capacity (ECEC) (cmol$_+$/kg)	**Calculation: Sum of Ca,Mg,K,Na,Al,H (cmol$_+$/kg)	16	14	27	20
Calcium (%)	**Base Saturation Calculations - Cation cmol$_+$/kg / ECEC x 100	7.7	19	7.2	9.1
Magnesium (%)		12	31	20	23
Potassium (%)		1.6	2.8	1.2	1.8
Sodium - ESP (%)		6.7	17	28	32
Aluminium (%)		21	5.8	10	6.6
Hydrogen (%)		51	24	33	28
Calcium/Magnesium Ratio	**Calculation: Calcium / Magnesium (cmol$_+$/kg)	0.62	0.61	0.35	0.40
Zinc (mg/kg)	Rayment & Lyons 2011 - 12A1 (DTPA)	<0.5	1.8	2.0	3.0
Manganese (mg/kg)		2.2	7.5	9.0	8.6
Iron (mg/kg)		190	172	117	143
Copper (mg/kg)		0.14	0.74	<0.1	0.74
Boron (mg/kg)	**Rayment & Lyons 2011 - 12C2 (Hot $CaCl_2$)	0.61	1.2	1.9	1.7
Silicon (mg/kg Si)	**Inhouse S11 (Hot CaCl2)	44	43	53	52
Total Carbon (%)	Inhouse S4a (LECO Trumac Analyser)	3.1	2.2	3.4	2.0
Total Nitrogen (%)		0.20	0.14	0.21	0.20

Carbon/Nitrogen Ratio	**Calculation: Total Carbon/Total Nitrogen	15	15	16	9.7
Basic Texture	**Inhouse S65	Clay	Clay	Clay	Clay Loam
Basic Colour		Brownish	Brownish	Brownish	Brownish
Chloride Estimate (equiv. mg/kg)	**Calculation: Electrical Conductivity x 640	132	335	1,409	1,141
Total Calcium (mg/kg)	Rayment & Lyons 2011 - 17C1 Aqua Regia	482	705	765	635
Total Magnesium (mg/kg)		1,486	1,404	1,634	1,207
Total Potassium (mg/kg)		1,000	1,064	977	990
Total Sodium (mg/kg)		346	621	1,859	1,486
Total Sulfur (mg/kg)		470	667	1,928	1,153
Total Phosphorus (mg/kg)		393	297	420	253
Total Zinc (mg/kg)		38	31	39	23
Total Manganese (mg/kg)		70	68	73	51
Total Iron (mg/kg)		17,834	17,622	16,764	11,879
Total Copper (mg/kg)		13	10	13	8.1
Total Boron (mg/kg)		4.1	4.6	6.8	5.7
Total Silicon (mg/kg)		1,000	890	956	877
Total Aluminium (mg/kg)		21,172	16,052	19,578	14,014
Total Molybdenum (mg/kg)		0.84	1.5	1.3	1.0
Total Cobalt (mg/kg)		3.0	3.1	3.2	2.1
Total Selenium (mg/kg)		1.3	0.91	1.5	0.74
Total Cadmium (mg/kg)		<0.5	<0.5	<0.5	<0.5
Total Lead (mg/kg)		27	20	21	17
Total Arsenic (mg/kg)		5.3	4.7	6.8	4.6
Total Chromium (mg/kg)		17	14	15	13
Total Nickel (mg/kg)		7.9	6.9	9.0	5.1
Total Mercury (mg/kg)		<0.1	<0.1	<0.1	<0.1
Total Silver (mg/kg)		<1	<1	<1	<1
Labile Carbon (%)	**Blair 1995 - 0.333 M Potassium Permanganate	0.64	0.27	0.68	0.57

1. All results presented as a 40°C oven dried weight. Soil sieved and lightly crushed to < 2 mm.

2. Methods from Rayment and Lyons, 2011. Soil Chemical Methods - Australasia. CSIRO Publishing: Collingwood.

3. Soluble Salts included in Exchangeable Cations - NO PRE-WASH (unless requested).

4. 'Morgan 1 Extract' adapted from 'Science in Agriculture', 'Non-Toxic Farming' and LaMotte Soil Handbook.

5. Guidelines for phosphorus have been reduced for Australian soils.

6. Indicative guidelines are based on 'Albrecht' and 'Reams' concepts.

7. Total Acid Extractable Nutrients indicate a store of nutrients.

8. National Environmental Protection (Assessment of Site Contamination) Measure 2013,

Schedule B(1) - Guideline on Investigation Levels for Soil and Groundwater. Table 5-A Background Ranges.

9. Information relating to testing colour codes is available on sheet 2 - 'Understanding your agricultural soil results'.

10. Conversions for 1 cmol+/kg = 230 mg/kg Sodium, 390 mg/kg Potassium,

11. Conversions to kg/ha = mg/kg x 2.24

12. The chloride calculation of Cl mg/L = EC x 640 is considered an estimate, and most likely an over-estimate

Memo A1.

Centre for Tropical Water and Aquatic Ecosystem Research

TropWATER

JAMES COOK UNIVERSITY AUSTRALIA

Technical Memorandum - Resinex NR-1 Ion-Exchange Resin Nitrate Recovery Trials

Michelle Tink & Adam Canning
February 2023

Overview

Resinex NR-1 is a high purity, premium grade, crosslinked polystyrene divinylbenzene resin that is highly selective for nitrate ions, even with high background sulphate. Given that resin has a strong bind with nitrate, not all nitrate can be recovered by standard pre-testing extraction procedures, and calibration adjustments are required to attain a more accurate estimate of nitrate bound to resin. This study aimed to quantify the relationship between nitrate applied to resin and the quantity recovered, as this relationship can then be used to calibrate estimates for future samples using Resinex NR-1.

Method

Twenty-two sterilised pottles containing 50g of Resinex NR-1 were spiked with various quantities of nitrate-N, delivered as potassium nitrate (KNO_3) dissolved in 100 mL of milli-Q water. Nitrate spikes included three replicates of the following quantities: 0, 18, 36, 66, 88, 109, and 131 mg NO_3^--N, along with an additional single trial of 175 mg NO_3^--N. Spiked samples were agitated for 1h at 300 rpm, and then left for 24h for nitrate to bind to resin prior to extraction and recovery testing.
To extract nitrate from the resin, the supernatant was drained from the resin and resin then mixed with 200 mL of 2M KCl for 2h at 300 rpm. The nitrate within the extractant was then tested using spectrophotometry and total quantity of nitrate-N quantified.

Results

There was an almost perfect correlation between the quantity of nitrate-N added to the resin and the quantity of nitrate-N recovered (R^2=0.99; Fig. A1.), allowing for reliable estimates of resin nitrate following a single extraction and calibration.

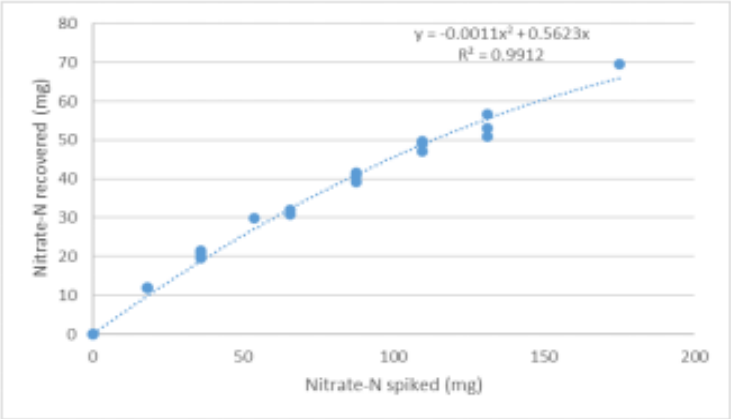

Fig. 1. The relationship between the nitrate-N spiked and then recovered from Resinex NR-1 using a single extraction with 200 mL of 2M KCl.