Nitrogen fertilizer dose alters fungal communities in sugarcane soil and rhizosphere

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Fungi play important roles as decomposers, plant symbionts and pathogens in soils. The structure of fungal communities in the rhizosphere is the result of complex interactions among selection factors that may favour beneficial or detrimental relationships. Using culture-independent fungal community profiling, we have investigated the effects of nitrogen fertilizer dosage on fungal communities in soil and rhizosphere of field-grown sugarcane. The results show that the concentration of nitrogen fertilizer strongly modifies the composition but not the taxon richness of fungal communities in soil and rhizosphere. Increased nitrogen fertilizer dosage has a potential negative impact on carbon cycling in soil and promotes fungal genera with known pathogenic traits, uncovering a negative effect of intensive fertilization.

The rhizosphere, the interface between soil and roots, is a biologically active zone where roots and microorganisms interact1. The identity of microorganisms in the rhizosphere has great influence on plant vigour and growth with beneficial, neutral and detrimental microbial relationships2. Structural and functional diversity of rhizosphere microbial populations in natural and agricultural systems is affected by plant species, crop cultivar, phenology, soil type, agronomy practice and other environmental factors3–5.

It is estimated that global N fertilizer use will increase threefold by 2050 to meet the growing need for food6. The use of chemical fertilizers is often accompanied by inefficiencies that result in pollution and soil degradation7. The type and quantity of N fertilizer affects physical, chemical and biochemical properties of soil8,9, as well as bacterial and arbuscular mycorrhizal fungal (AMF) communities in the rhizosphere10,11.

Increasing the dose of NPK fertilizer has been associated with an increased presence of bacteria and fungi in crop soils12. However, whether the dose of fertilizer modifies rhizosphere fungal communities is largely unknown, with the exception of AMF which diminish in taxon richness in maize roots in response to N fertilizer11. The objective of this study was to investigate how N fertilizer rates influence fungal communities in rhizosphere and soil of a commercial sugarcane crop system.

Results and Discussion

We sampled a total of 822 operational taxonomic units (OTUs) (February) and 820 OTUs (November) of fungi from 1135428 (February) and 1187048 (November) pyrosequence reads, respectively. Irrespective of collection time, the Chao1 metric showed no difference in species richness between low and high N treatment: in February 18.6 ± 8.83 (high N) and 17.2 ± 9.72 (low N); in November 19.3 ± 8.57 (high N) and 18.7 ± 9.63 (low N). Similar to the Chao1 metric, Simpson’s index confirmed no difference in species richness: in February 0.768 ± 0.162 (high N) and 0.773 ± 0.125 (low N); in November 0.787 ± 0.126 (high N) and 0.782 ± 0.133 (low N). However, while fungal taxon richness did not differ between N-fertilizer doses, clear differences were detected in the fungal community composition in sugarcane rhizosphere and soil at each sampling time (Fig. 1).

Sequence-based community profiling is increasingly adopted to study plant root-associated bacterial and fungal communities14,15 as this approach circumvents culture bottlenecks. Species richness can be estimated from read counts of the internal transcribed spacer (ITS) region, but in the case of fungal communities these estimates
may be biased due to the differing lengths of the ITS in different fungal species\textsuperscript{16}, as shorter regions are preferentially amplified\textsuperscript{17}. However, this bias does not significantly alter estimates of relative abundance (species evenness) of the dominant OTUs\textsuperscript{17,18} and is expected to be even further attenuated in comparisons of relative abundance within a community, e.g. between treatments. Our results (Fig. 1), based on the relative abundance of dominant OTUs and showing compositional dissimilarities between sugarcane soil and rhizosphere fungal communities associated with N fertilizer dose, are therefore unlikely to be significantly affected by this amplification bias, and detected community dissimilarities would reflect true biological variation.

Interesting results are apparent even at the phylum level. Across all soil and rhizosphere samples collected in February and November, fungal taxonomic diversity involves mainly two phyla, Ascomycota and Basidiomycota (Fig. 2). The relative abundance of Ascomycota was generally higher in high N fertilizer dose conditions compared to low N, whereas for Basidiomycota it was lower. Consistent with this result, most saprotrophic microfungi are Ascomycota\textsuperscript{19} and their growth rate is correlated with N availability\textsuperscript{20}. Basidiomycetes are
widely recognised as lignin decomposers and thus important for carbon cycling in soil; in the same way, this beneficial function could be adversely affected by high N dose (Fig. 2). In agreement to our results, deleterious effects of mineral fertilizers on soil and plant function has been proposed because it negatively impacts on symbiotic relationships, including diazotrophic and AM symbioses.

To identify the known fungal genera that were most-altered in relative abundance by N fertilizer doses, we compared the relative abundances of identified OTUs in rhizosphere and soil between low N and high N doses, using the ratio (low N/high N) as a means of evaluation (Tables 1 and 2). The data show that in all samples, the genera positively or negatively affected include groups known for their positive impact on soil and plant health (biocontrol, decomposers) or to the contrary, for their negative impact as plant pathogens. For example, irrespective of collection time, the genera positively or negatively affected include groups known to influence plant health. Further research should elucidate the specific roles of these fungal taxa in the rhizosphere to reduce the need for agrochemicals, reduce disease incidence and improve crop performance. To advance the ecological management of crop soils, understanding is needed of how beneficial microbial relationships can be fostered.

### Methods

#### Sample collection

We sampled three individual plots within a 4-hectare field trial in the Burdekin region, Australia (near Ayr, S19°43.95', E141°710.727', 26 m above sea level). The soil is a silty-clay loam. Within each plot, half the sugarcane crops received an N supply rate of 200 kg N ha⁻¹ (recommended 'high' N in the form of urea) while the other half received 40 kg N ha⁻¹ (low N). From the three replicate plots, six bulk-soil and six roots with adhering soil (constituting the rhizosphere samples in our study) biological replicates were sampled at 0–10 cm depth from sugarcane receiving either N supply rate. Sampling was performed on a first and second ratoon crop of three sugarcane cultivars (Australian cultivars Q208 and Q186, and Brazilian cultivar S79-2313) for a total of 144 root and bulk soil samples. Samples were immediately placed in a cool box for 2 days during transport to the laboratory and stored at −20 °C for isolation of DNA. Sampling was carried out in February 2012 and November 2012 (4 and 3 months after fertilizer application, respectively) to assess the reproducibility of the observations.

#### DNA extraction and pyrosequencing

Total DNA was extracted from soil and rhizosphere samples using Mo Bio PowerSoil DNA isolation kits following manufacturer’s instructions (Mo Bio Laboratories, Inc., Carlsbad, CA, USA). To profile fungal communities, the fungal internal transcribed spacer (ITS) region was PCR-amplified from isolated soil and rhizosphere DNA using ITS1F (5' -

**Table 1** Fifteen fungal OTUs whose relative proportion in the community was increased or decreased the most between low and high N fertilizer dose. Ratio denotes the relative abundance of OTUs in low N compared to high N. The samples were from February 2012 collection.

| Rhizosphere | Soil |
|-------------|------|
| OTU         | Genus   | Function Ref | Ratio | OTU         | Genus   | Function Ref | Ratio |
| 110         | Clonostachys | Biocontrol     | 31 | 41.8 | 175         | Unidentified | n/a | 21 |
| 178         | Agrocybe   | Decomposer     | 32 | 26.3 | 110         | Clonostachys | Biocontrol | 31 | 14.2 |
| 123         | Emericellipsoid | Biocontrol     | 33 | 26  | 82          | Calcarisporiella | n/a | 14 |
| 205         | unidentified | n/a            | 34 | 24.3 | 207         | Waira | n/a | 13.3 |
| 183         | Clotopilus  | Biocontrol     | 35 | 23.7 | 199         | unidentified | n/a | 13 |
| 212         | Resinicum   | Decomposer, biocontrol | 26,36 | 16.2 | 43          | Epicoccum | Biocontrol | 36 | 11.3 |
| 145         | Kanasus     | n/a            | 37 | 11.8 | 253         | unidentified | n/a | 10.2 |
| 180         | Conocybe    | Biocontrol     | 38 | 11.2 | 250         | unidentified | n/a | 7.7 |
| 126         | Sarocladium | Pathogen       | 39 | 7.3  | 151         | Scedosporium | n/a | 6.7 |
| 154         | Cynauscas   | Decomposer     | 40 | 6    | 243         | unidentified | n/a | 6.6 |
| 199         | unidentified | n/a            | 41 | 5.5  | 180         | Conocybe | Biocontrol | 37 | 6.6 |
| 176         | unidentified | n/a            | 42 | 5.3  | 205         | unidentified | n/a | 5.7 |
| 204         | Ceratobasidium | Biocontrol     | 43 | 5.3  | 216         | unidentified | n/a | 5.3 |
| 1            | Aporosporella | n/a            | 44 | 4.9  | 97          | Dactyliella | Biocontrol | 41 | 3.3 |
| 67          | Emericellus | Pathogen       | 45 | 4.6  | 76          | Spiromystix | n/a | 3.8 |
| 136         | Nectria     | Pathogen       | 46 | 0.3  | 196         | unidentified | n/a | 0.4 |
| 160         | Zophella    | n/a            | 47 | 0.3  | 89          | Scolocobasidium | n/a | 0.4 |
| 48          | Preussia    | Biocontrol     | 48 | 0.04 | 129         | Trichothecium | Pathogen | 45 | 0.05 |
| 161         | unidentified | n/a            | 49 | 0.07 | 126         | Sarocladium | Pathogen | 38 | 0.07 |
| 113         | Cordyceps  | Biocontrol     | 50 | 0.07 | 258         | Mortierella | Biocontrol | 47 | 0.09 |
| 4           | Capnadium  | Pathogen       | 51 | 0.15 | 200         | unidentified | n/a | 0.3 |
| 194         | Coprinopsis | n/a            | 52 | 0.17 | 178         | Psilocybe | n/a | 0.10 |
| 114         | Metarhizium | Biocontrol     | 53 | 0.17 | 143         | Arthrinium | Biocontrol | 50 | 0.12 |
| 108         | unidentified | n/a            | 54 | 0.18 | 187         | unidentified | n/a | 0.14 |
| 93          | Scytalidium | n/a            | 55 | 0.21 | 186         | Vassellum | n/a | 0.20 |
| 44          | Euxerophilium | Decomposer, biocontrol, pathogen | 56 | 0.21 | 64          | Thermoascus | Decomposer | 52 | 0.20 |
| 97          | Dactyliella | Biocontrol     | 57 | 0.22 | 122         | Acronemum | n/a | 0.20 |
| 236         | Dioszezia  | n/a            | 58 | 0.23 | 236         | Dioszezia | n/a | 0.22 |
| 59          | Rhinolodiella | n/a            | 59 | 0.25 | 111         | unidentified | n/a | 0.22 |
| 140         | Ophiocordycipes | Biocontrol     | 60 | 0.27 | 191         | Pluteus | n/a | 0.24 |

In summary, our findings add to understanding on how different doses of N influence fungal communities. The data show that changes in relative abundance of fungal population in response to N doses are not restricted to AMF but span a wide range of fungal taxa, including genera known to influence plant health. Further research should elucidate the specific roles of these fungal taxa in sugarcane rhizosphere and soils, and on the health of the plant. It is an attractive concept to manipulate the microbial community in the rhizosphere to reduce the need for agrochemicals, reduce disease incidence and improve crop performance. To advance the ecological management of crop soils, understanding is needed of how beneficial microbial relationships can be fostered.
CTTGGTCATTAGGTAA-3’) and ITS2B (5’-GCTGCGTTCTTCATCGATGC-3’) primers modified on the 5’ end to contain the 454 FLX Titanium Lib L adapters. A unique MID was used for each sample to identify sequencing reads to

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