Effects of the invasive grass *Andropogon gayanus* Kunth (gamba grass) on soil nitrogen dynamics and the soil microbial community

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Summary The African grass *Andropogon gayanus* Kunth (gamba grass) is a serious threat to savanna ecosystems across northern Australia. The success of this high biomass grass, growing on low nitrogen (N) status savanna soils, may, in part, be due to the documented ability of gamba grass to alter the soil microbial community via allelopathy. Suppressing the activity of ammonium oxidising community reduces nitrification and conserves N in the ecosystem. This mechanism could play a key role in the invasion success and competitive superiority of gamba grass in Australian savannas.

We examined the soil ammonia oxidiser (archaea and bacteria), and N-fixing communities following invasion, as well as soil N relations. Soil inorganic N availability (NO_3^- and NH_4^+) under native grass and gamba grass stands were not significantly different. This was reflected in the microbial communities, with no difference in *amoA* gene copy number between the native and gamba grass soils. While gene abundances were similar, the identities of some *amoA* phylotypes between native grass and gamba grass soils were different. The N-fixing bacterial community, as measured by *nifH* amplicons sequencing, provided new information about N-fixing bacteria in these soils, however there were no measurable differences between native and gamba grass soils. The dominant N fixers were *Streptomyces*, *Actinomyces*, *Candidatus, Methylomirabilis* and *Pseudomonas.*

Keywords *Andropogon gayanus*, ammonia oxidiser, exotic grasses, invasive alien grasses, nitrogen cycling genes, nitrification, savanna.

INTRODUCTION

Gamba grass (*Andropogon gayanus* Kunth) invasion is a key threat to savanna ecosystems across northern Australia. Gamba grass dramatically transforms the structure and function of invaded ecosystems; producing 10 times more biomass than native grasses; altering fires regimes, water, nutrient and carbon cycling, and increasing savanna tree mortality (Rossiter *et al.* 2003, Rossiter-Rachor *et al.* 2008, 2009, Setterfield *et al.* 2010, 2013). These impacts contributed to gamba grass being declared a weed across all northern states of Australia (in 2008), declared a Key Threatening Process under the EPBC Act (in 2010), and being declared a weed of national significance (WoNS; in 2012). Despite these declarations, gamba grass is now widely distributed throughout native savannas, with the invaded area expanding rapidly, covering approximately 10,000–15,000 km^2 in the Northern Territory (Adams and Setterfield 2016).

The paradox of a high biomass grass growing on low N status soils has led researchers to question if gamba grass productivity could be sustained in the long-term, or would heavily invaded sites experience a 'run down' or decline in soil N, resulting in reduced plant vigour, and productivity. While there is no evidence of long-term declines in total soil N pools on gamba grass sites, invasion has been associated with significant increases in soil ammonium availability, accompanied by decreases in soil nitrate availability, compared to native grasses (Rossiter-Rachor *et al.* 2009). These results led to speculation that: (1) gamba grass may inhibit the process of nitrification, as it does in its native range in Africa, by altering the soil microbial community, and/or (2) gamba grass may stimulate N fixation, again by altering the soil microbial community (Rossiter-Rachor *et al.* 2009).

Gamba grass is one of several African grasses known to release allelopathic nitrification inhibitors from their roots, which suppress the activity of ammonium oxidising bacteria (Lata *et al.* 2004, Subbaro *et al.* 2012). Nitrification (the oxidation of ammonium to nitrate) is a two-step process. The first step being mediated by ammonia oxidisers (AO), comprising ammonia oxidising bacteria (AOB) and archaea (AOA). Suppressing this process, and retaining N in the ecosystem as ammonium reduces the potential for N losses via leaching, and denitrification (Subbaro *et al.* 2012). The capacity of plants to inhibit nitrification is likely to be an adaptation mechanism for the

conservation and efficient use of N in ecosystems with low N availability, such as savannas (Abbadie and Lata 2006). Field experiments have shown that grasses that inhibit nitrification exhibit greater productivity in above-aground biomass than those that lack this ability (Lata *et al.* 2004). We speculate this mechanism could play a key role in the invasion and persistence of gamba grass in the low N savannas of Australia, as it could allow gamba grass to regulate soil N relations in the invaded ecosystem and outcompete native species for N (Rossiter-Rachor *et al.* 2009). It is therefore *critical* that we understand the impact of gamba grass invasion on soil microbial communities, particularly those involved in nitrification (ammonium oxidising bacteria or archaea), and N fixation (N-fixing bacteria). We aimed to quantify changes in the soil microbial community that occur in response to gamba grass invasion, and determine if altered microbial communities were accompanied by changes in soil N relations.

MATERIALS AND METHODS

Study site Soil microbial communities and soil N were sampled at three paired plots near Batchelor, approximately 100 km south of Darwin, Northern Territory, Australia. The overstorey was dominated by *Eucalyptus miniata* (Cunn. ex Schauer) and *E. tetrodonta* (F.Muell), with a grass understory dominated by native perennial grasses *Alloteropsis semialata* (R.Br.) Hitchc. and perennial *Sorghum* spp. Each paired plot consisted of one native grass plot, and an adjacent gamba grass invaded plot.

Sample collection Soil samples were collected in April 2015 at the end of the wet/growing season. Six replicate soil cores (7.5 × 15 cm) were taken within each native and gamba grass plot. Soil cores were placed in a snap-lock polyethylene bag and stored on ice until return to the lab, where approximately 0.25 g of soil was taken for nucleic acid extraction. Soil genomic DNA was extracted using a 50 prep PowerLyzer PowerSoil DNA Isolation Kit (MB-12855-50) according to the manufacturer's instructions. A subsample of soil was taken for determination of gravimetric moisture content (weighed, dried at 105°C for 48 h, and reweighed). The remainder was dried at 40°C for 48 h, ground, and analysed for Total N (% N), NO_3^- and NH_4^+ (mg kg^{-1}) on a Carlo Erba analyser.

Soil microbial community The soil ammonia oxidising community in native and gamba plots was measured using two approaches. The first approach was a qPCR analysis whereby DNA was extracted from soil and a portion of the ammonia oxidizing gene from archaea (A-*amoA*) and bacteria (B-*amoA*) was amplified in separate experiments and gene copy numbers measured (following Abell *et al.* 2011). These data provide a relative measure of ammonia oxidation gene abundance in native and gamba grass soil.

The second approach was a microarray analysis whereby ammonia oxidizing gene probes were used in hybridisation assays (following Abell *et al.* 2012). The bank of probes used in these assays provides a broader measure of the diversity of ammonia oxidisers in this system, than is possible by qPCR. This is a semi-quantitative approach which can be used to measure patterns and compare the ammonia oxidizer community in native versus gamba grass soils.

For the N-fixing community we used next generation sequencing targeting the N-fixing (*nif*) gene. These data give a semi quantitative measure of abundance in the form of sequence counts and they provide a measure of diversity so we can determine what N-fixers are dominant in these soils.

Soil N availability We measured soil inorganic N availability *in situ* in native and gamba grass plots using mixed ion-exchange resin bags (Dowex-MR3; Sigma, St. Louis, Missouri, USA) following Rossiter-Rachor *et al.* (2009). These data provide an index of plant NH_4^+ and NO_3^- availability, and were related back to the microbial communities present.

RESULTS AND DISCUSSION

Ammonia oxidising gene copy number – qPCR Ammonia oxidation in this system was overwhelmingly dominated by archaea ammonia oxidisers (AOA) with 16 times more archaeal oxidisers than bacterial nitrifiers (AOB) (Table 1).

The MDS of communities based on total gene copy number as measured by qPCR (irrespective of the identity of the ammonia oxidisers) showed no clear pattern between native and gamba grass soils (Figure 1).

Table 1. Gene copy numbers of archaeal nitrifiers (AOA) and bacterial nitrifiers (AOB) in native grass and gamba grass plots (mean ± standard deviation; n = 6).

Plot type	AOA gene copy numbers per ng DNA	AOB gene copy numbers per ng DNA
Native grass	14,923 ± 642	894 ± 52
Gamba grass	14,979 ± 699	887 ± 50

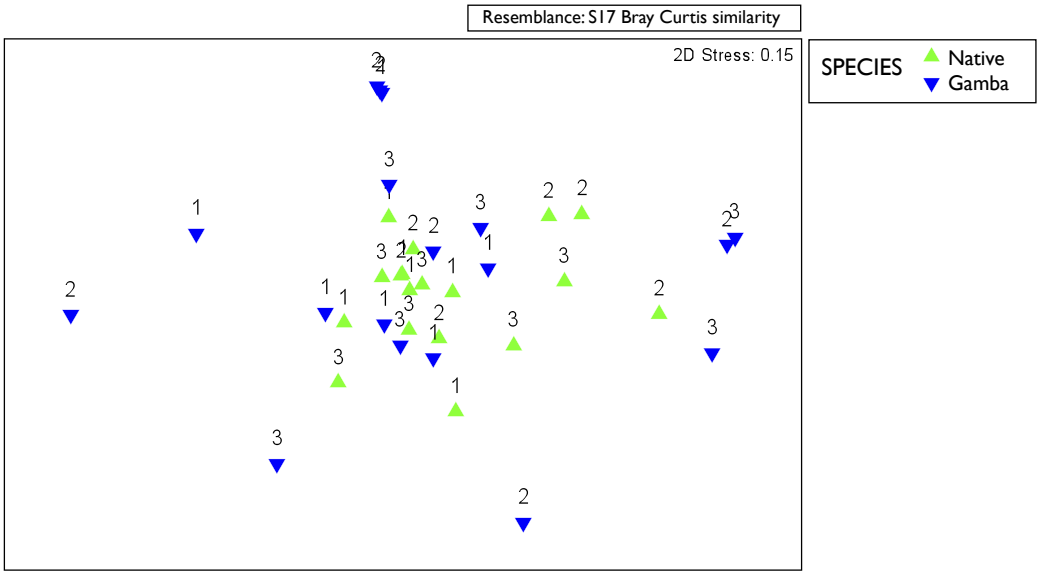

Figure 1. MDS plot of the ammonia oxidising microbial community size in native and gamba grass plots (A-*amoA* and B-*amoA* gene copy numbers per plot). Replicates not pooled.

Ammonia oxidising gene diversity – microarray The MDS of communities based on the presence or absence of AOA phylotypes derived from the microarray probes (a measure of genetic diversity of the AOA genes, Figure 2) showed no clear pattern between native and gamba grass plots. In fact, no significant community level patterns were measured between habitats due to the large sample variability, particularly

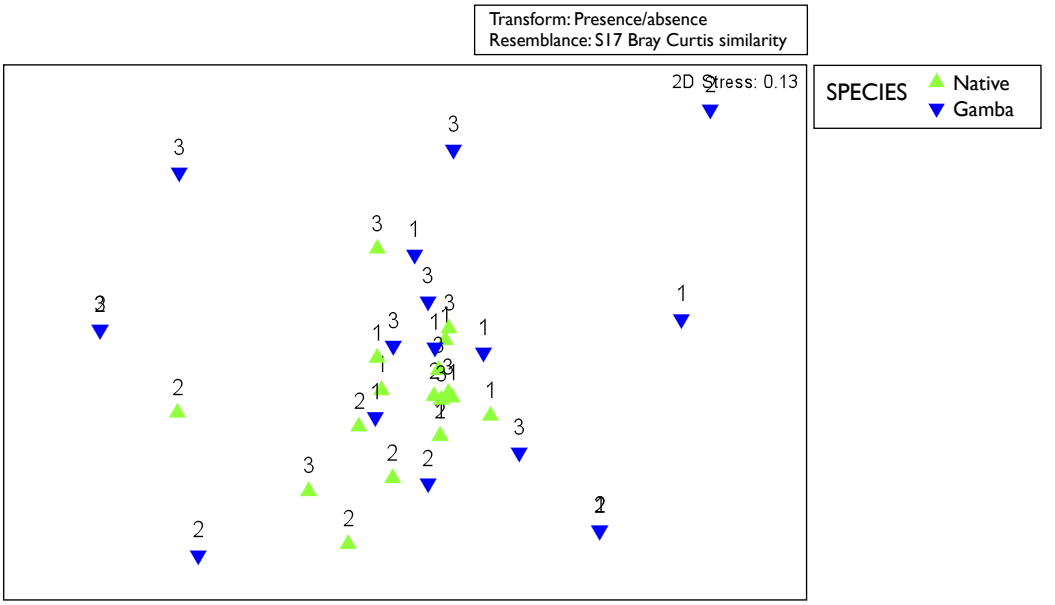

Figure 2. MDS plot of the archaeal ammonia oxidiser (AOA) community structure in native and gamba grass plots (ANOSIM global R >0.05).

within gamba samples. Despite this, the MDS showed that some gamba samples were separate and therefore different from the main cluster (Figure 2). This suggests that while there may not be any major differences compared to native, there are some unique aspects of the AOA community for some gamba samples.

The microarray data showed that the DNA from both native and gamba grass plots hybridized to 45 AOA genes across all samples; 21 distinct to gamba plots, eight distinct to native plots and 16 common to both native and gamba plots (Figure 3).

N-fixing community composition derived from the next generation sequencing nifH gene data After removing rare sequences, eleven N-fixing genera were detected across most samples (data not shown). Only one genus (*Shinella*) was unique to gamba plots. *Streptomyces*, *Actinomyces*, *Candidatus Methylomirabilis* and *Pseudomonas* were dominant across all sites.

Soil N There was no significant differences in soil N parameters between native and gamba grass plots ($P > 0.05$; Table 2). Our results directly contrast with a previous study by Rossiter-Rachor *et al.* (2009), at a different savanna location, which found significantly increased soil ammonium availability, and decreased soil nitrate availability, as a result of gamba grass invasion. We speculate that the difference between the two studies may be due to differences in soil conditions, or the age of the gamba grass tussocks and their root density. Lata *et al.* (2000, 2004) found that the impact of the African grass *Hyparrhenia diplandra* on nitrification varied significantly with site, and also root density, and did not always result in a depressive effect on nitrification.

In conclusion, soil N in native and gamba grass plots was not significantly different, and this was reflected in the microbial communities. There was no evidence of higher ammonia concentrations in gamba grass soils. Subsequently, there was no evidence for suppression of ammonia oxidation as measured by *amoA* gene copy number. Although gene abundances were similar, there were differences in the identities of *amoA* phylotypes between native and gamba grass soil. This is for subsequent investigations. There was also no evidence for stimulation of N fixation as measured by *nifH* sequence counts. N fixers dominant in these systems: *Streptomyces*, *Actinomyces*, *Candidatus, Methylomirabilis* and *Pseudomonas.*

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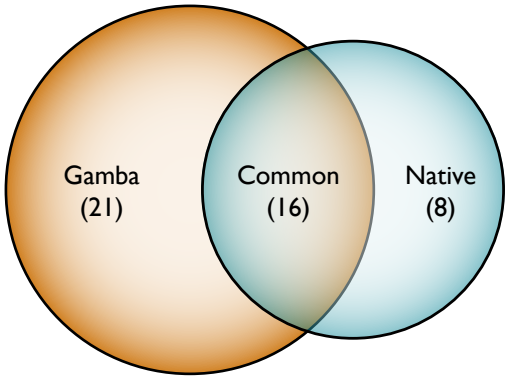

Figure 3. Venn diagram illustrating the number of the ammonia oxidising archaeal genes (a proxy for ammonia oxidising archaea) detected within and between each of the grass types.

Table 2. Soil chemical parameters in native and gamba grass plots (mean ± SE).

Soil chemistry	Native grass soil	Gamba grass soil
Gravimetric water content (%)	6.25 (0.16)	6.58 (0.15)
NH_4–N (mg kg^{-1})	1.77 (0.10)	1.70 (0.11)
NO_3–N (mg kg^{-1})	0.28 (0.04)	0.27 (0.05)
Total N (% N)	0.10 (0.005)	0.09 (0.004)
Ammonium availability (ng NH_4^+ g^{-1} resin day^{-1})	972 (16)	1068 (40)
Nitrate availability (ng NO_3^- g^{-1} resin day^{-1})	1177 (98)	1055 (118)

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