The effect of ecosystem engineers on N cycling in an arid agroecosystem

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Abstract
Ecosystem engineers—such as earthworms, termites and ants—are an important component of soil biodiversity and have been shown to contribute to aboveground productivity in native and managed ecosystems. Although their role in physical alteration of soils is appreciated, less is known about their effect on soil nutrient cycling, particularly in arid systems where termites and ants are the dominant ecosystem engineers. We explored the effect of termite reduction and tillage on soil nitrogen (N) biogeochemistry in soils from the northeasternmost wheat growing region in Western Australia. We assessed total soil N, potentially mineralizable N, and dissolved N pools, as well as soil N fluxes, such as proteolysis and N mineralization. We predicted that soils with native termite and ant populations would have greater N pools and rates of transformations between pools. While we found that many soil N pools were up to 2.5 times larger with native termite populations (e.g. dissolved organic N, NH_4^+), we found that the rate of transformations between pools was reduced relative to the reduced termite plots. While the reason behind this trend needs further exploration, the larger soil N pools in sites with native levels of ecosystem engineers implies that the conservation of soil macrofauna, particularly those that translocate N through the soil profile, may be important in the sustainable management of cropped lands.

Key Words
ecosystem engineer, termites, soil nitrogen, organic nitrogen, conservation agriculture, soil biogeochemistry

Introduction
Ecosystem engineers, such as earthworms, ants, and termites, are beneficial to soil health and ecosystem productivity in both native and managed systems (Lavelle et al. 2006; Brussaard et al. 2007; Evans et al. 2011). In temperate systems, earthworms act as the dominant ecosystem engineers (Wall et al. 2015), while ants and termites fill this niche in semi-arid and arid regions. Until recently, the effect of termites and ants in agricultural systems was largely unknown, with the exception of subsistence farming systems in Africa (Black & Okwakol 1997; Sileshi et al. 2009). Evans et al. (2011) showed that sites with larger ant and termite populations had greater crop yield in a wheat-fallow system in northeastern Western Australia. They found that with termite and ant populations intact, there was greater water infiltration and greater mineral N availability. Their work suggested that 'conservation agriculture' practices—which are typified by lessened soil disturbance relative to conventional agriculture, thereby allowing ants and termites to thrive—can increase crop yield and potentially reduce nitrogen fertilizer needs without decimating soil N stocks.

Although the role of ants and termites as "engineers" that change the physical landscape in soils is appreciated (Jouquet et al. 2006; Brussaard et al. 1997; Jones et al. 1994), less is known about how they alter the biogeochemistry of the soils they inhabit. In addition to observing higher soil mineral N in plots with greater numbers of termites and ants, Evans et al. (2011) also found N-fixing bacteria in the hindgut of the termites. But, whether N transformations mediated by the free-living microorganisms in these soils contributed to the different sizes of N pools is unknown.

Our objective for this study was to assess the size of soil N pools and the magnitude of fluxes between pools, in order to determine the effect of termites on the processes of free-living soil microorganisms. In addition, we determined the effect of shallow tillage on soil N pools and fluxes. We hypothesized that termites and ants, which act as ecosystem engineers in arid environments, systematically alter the soil N cycle by increasing both the amount of N-containing compounds (SOM, mineral N produced through N fixation) and by stimulating the activity of the free-living microbial community to process N containing compounds at a higher rate.

Methods

Field site and soil sampling

We obtained soils from the study of Evans et al. (2011), in which a two-way factorial design was used to assess the effect of soil macroinvertebrate reduction and shallow tillage (for weed management) on wheat yield. Macroinvertebrate (mostly ants and termites) reduction was achieved though application of a synthetic pyrethroid insecticide (FMC Bi ex Ultra Emulsion, FMC), applied as per label instructions (3.75 g per 5 l water per m^2). The experiment was located in a wheat-fallow-wheat system 5 km from the northeastern limit of wheat farming in Western Australia (27.921°S, 115.028°E). The average minimum and maximum annual temperatures at the site were 13.4 °C to 27.4 °C and the mean annual precipitation was 284 mm. The site was managed conventionally from 1980 to 2000, after which conservation agriculture practices were implemented (reduced tillage, controlled traffic, and stubble retention). From 2000-2006, the abundance and diversity of ants and termites increased (Evans et al. 2011). In 2006, a two-way factorial experiment (insect exclusion/reduction and shallow tillage) was installed with 5 replicate plots of each treatment. In the current study, we used soils from the time of wheat harvest in the final year of the experiment (November 2008). Soils were sampled in 10 cm increments to 50 cm depth. Soils were stored at -20 °C and were shipped overnight on ice. Before weighing, we mixed soils and broke up any clods with a mortar and pestle.

Soil N pools and fluxes

We assessed soil N pools as soon as possible (within days) after the arrival of the soils. We assessed total soil N using dry combustion (LECO Tru-Mac) of soils dried at 40 °C for 24 hrs. We extracted total dissolved N (TDN)—including dissolved organic N (DON) and mineral N (ammonium (NH_4^+) and nitrate (NO_3^-)) pools—using 0.5 M K_2SO_4, which we analyzed using colorimetry (Miranda et al. 2001; Mulvaney 1996). We assessed potentially mineralizable nitrogen (PMN) through the accumulation of NH_4^+ over 7 days under anoxic conditions (Curtin & Campbell 2008). We extracted NH_4^+ with 2 M KCl, which we analysed via colorimetry as above.

To acclimatize soil microorganisms, we pre-incubated soils in a dark, humid, 22 °C incubator at 30% WHC for 28 days before we determined potential rates of proteolysis and N mineralization. We assessed proteolysis as the accumulation of amino acids in a soil-citrate buffer slurry inhibited with toluene over 6 hours (Hofmockel et al. 2010), and determined amino acids concentrations with fluorimetry (Jones et al. 2002). We determined N mineralization by the accumulation of NH_4^+ and NO_3^- over 28 days at 50% WHC at 25°C; we extracted and analysed the concentration of mineral N with 2M KCl and via colorimetry.

Statistics

We performed a series of three-way ANOVA analyses (Sigmaplot 13.0) to identify the effect of the termite treatment, tillage and depth on the soil N pools and transformations. For the N transformations and PMN, where depth was significant in the three-way ANOVA models, we used two-way ANOVA at each depth to determine at which depths there was a significant effect of the treatments. Given the heterogeneous nature of the soil-invertebrate system, we adopted an indicative significance threshold of $p=0.10$.

Results

The effect of the reduction of termites and tillage was different for the various soil N pools. Total (combustible) soil N did not vary with either treatment, although depth was a significant effect ($p<0.001$) (Fig. 1a). The reduction of termites and the application of tillage resulted in significant differences in TDN (termites: $p=0.087$; tillage: $p=0.057$) (Fig. 1b). This resulted in a reduction by a factor of nearly two for each treatment, such that the soils subjected to both termite reduction and tillage had almost four times less TDN than soils with native termites and no tillage application. The TDN pool is comprised of DON, NH_4^+ and NO_3^-. The DON was 1.75 times greater for the soils with termites than the soils with reduced termites ($p<0.1$) (Fig. 1c). Similarly, the concentration of NH_4^+ was 2.5 times greater for the soils with the native populations of termites rather than the reduced ($p<0.05$) (Fig. 1d). The reduction of termites had no effect on the NO_3^- concentration, however soils that were tilled had half the amount of nitrate as soils that were not tilled ($p<0.05$) (Fig. 1e). PMN, a theoretical pool including the N available to be mineralized, was not affected by the termite reduction or tillage in a 3-way ANOVA model, but depth was nearly significant. However, when analysing the effect of termites and tillage for each depth separately, the effect of the termites and the interaction between the termites and tillage was significant ($p<0.1$) for the 0-10cm and 40-50cm depths. There were varying depth trends for the PMN pool under the different treatments, and the highest PMN was measured in soils with tillage and reduced termites (3.13 ug N/g dws) (Fig. 1f).

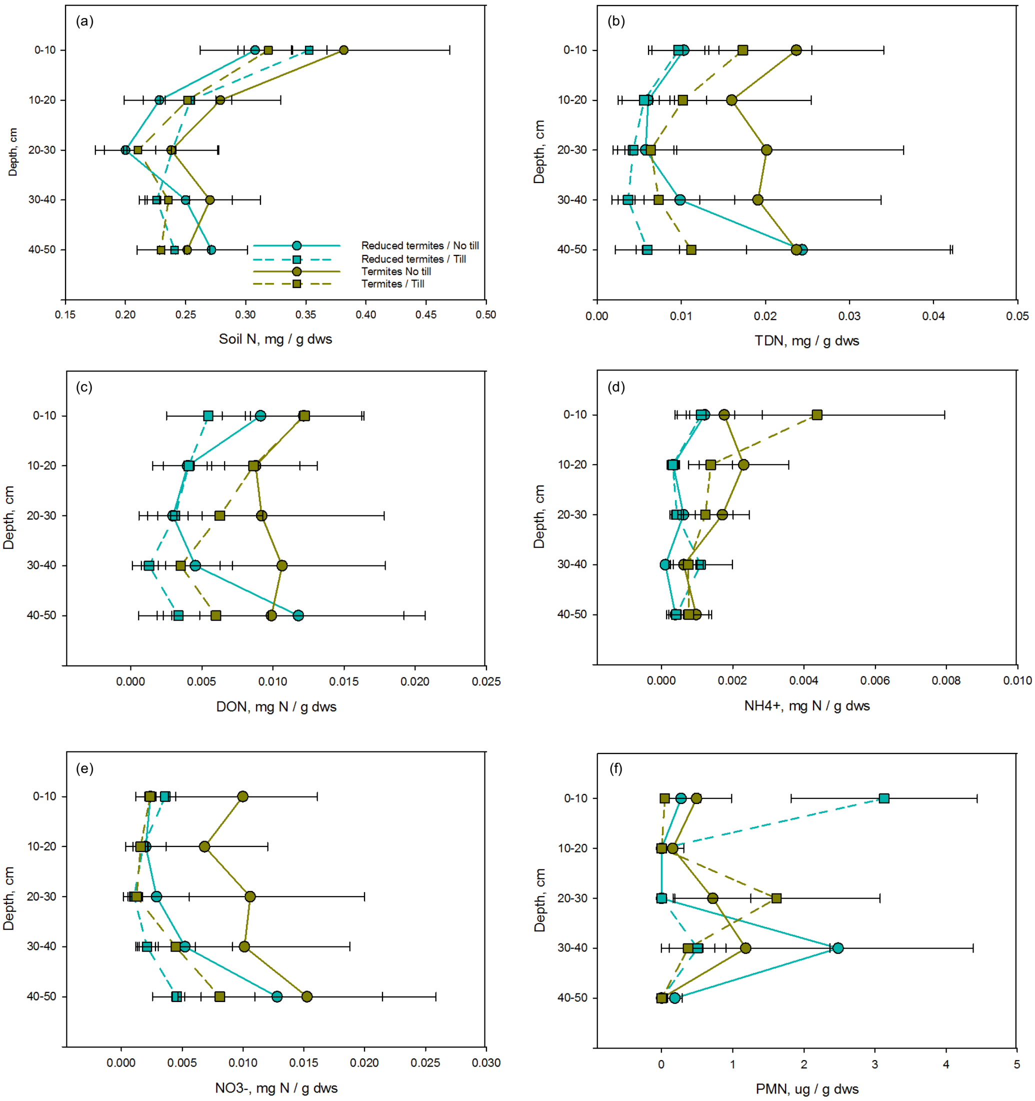

Figure 1. Total and soluble soil N pools. (a) Total Soil nitrogen, (b) TDN, (c) DON, (d) NH_4^+, (e) NO_3^-, and (f) PMN pools in soils under four crossed treatment levels: native termites, reduced termites, tillage, and no till.

The fluxes between N pools, as assessed through rate potential assays, were also affected by the reduction of termites and tillage (Fig. 2). Proteolysis, or the breakdown of proteins from the soil N or DON pools into amino acids, did not show a statistical effect of the termites and tillage treatment in a 3-way ANOVA. However, because depth was a significant factor ($p<0.001$), we further probed the effect of termites and tillage at each depth interval. At the 0-10 depth, the interaction between termites and tillage was highly significant (Fig. 2a, $p<0.05$). For the soils with the native termites, tillage reduced the proteolysis by 20% when relativized to total soil N ($p=0.065$). For soils with reduced termites, tillage resulted in a 1.8 times higher rate of proteolysis than in the untilled plots ($p=0.024$). The N mineralization potential, an assay which assesses the ability of the microbial community to transform N from soil organic matter and soluble pools to mineral N, was significantly affected by the reduction of termites, depth, and the interaction between termites and depth ($p<0.05$), as well we the three-way interaction between termite reduction, tillage, and depth ($p<0.1$) (Fig. 2b). To probe the direction and magnitude of the effect of the treatments at each depth, we again used two-way ANOVA. Termite reduction and the interaction between termites and tillage was significant ($p<0.1$) for the 0-10 cm depth. In the 0-10 cm depth, plots with reduced termites had over a 3 times greater N mineralization potential than plots with native termite populations ($p=0.06$). Similar to the proteolysis, tillage enhanced N mineralization for the termite reduction plots (till: 11.6 $\pm$ 2.9, no till: 5.9 $\pm$ 2.9), but hindered the process for the native termite plots (till: 0.2 $\pm$ 3.2, no till: 5.2 $\pm$ 2.9). Although not statistically different, both of the flux rate assays have an increase in the 20-30 depth that may be due to the translocation of nutrients by the termites and ants to the 'plastering' (a mixture of saliva and faeces) inside of the insect tunnel walls.

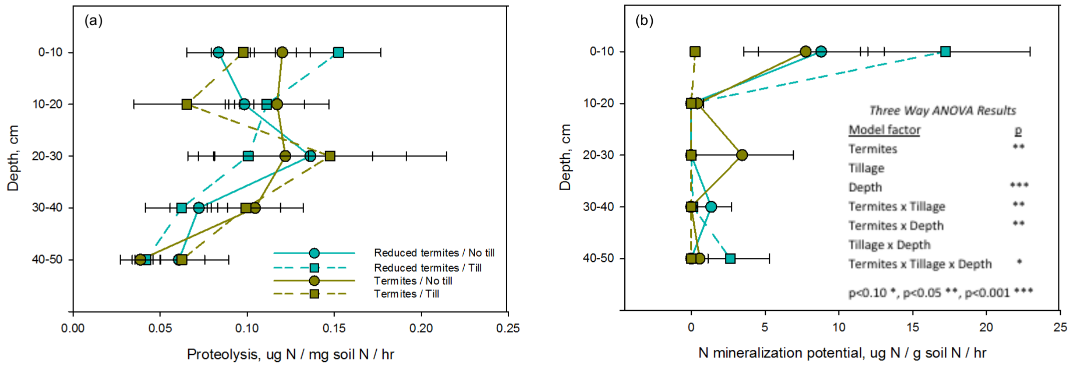

Figure 2. Fluxes of soil N between pools. (a) Proteolysis and (b) N mineralization potential are expressed on a per soil N basis.

Conclusion

We aimed to determine the effect of ecosystem engineers and tillage on soil N pools and fluxes. We found that plots with a greater abundance of termites and ants had higher DON and NH_4^+ pools than plots with reduced termites, which supported our hypothesis that ecosystem engineers enhance soil N pools. The fluxes into those pools (i.e. proteolysis, N mineralization) were largest for the plots with a reduction in termites, which did not support our hypothesis. However, this could be an artefact of accessibility differences in the field for plots with and without mechanisms for mixing resources (either by termite action or tillage). This is supported by the interaction between termite presence and tillage in the surface soils; N transformations were enhanced by tillage when the termites were reduced, but were hindered by tillage when termites were abundant. Further work will explore this dichotomy. However, the observation that organic N and NH_4^+ pools were enhanced by the presence of termites and ants—which likely contributed to the 36% increase in wheat yield observed previously by Evans et al (2011)—is promising, suggesting that managing soils to promote biodiversity can have positive environmental and economic benefits by reducing external N fertilization needs without yield trade-offs.

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