

Biological nitrification inhibition by weeds: wild radish, brome grass, wild oats and annual ryegrass decrease nitrification rates in their rhizospheres

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Abstract. This study investigated the ability of several plant species commonly occurring as weeds in Australian cropping systems to produce root exudates that inhibit nitrification via biological nitrification inhibition (BNI). Seedlings of wild radish (*Raphanus raphanistrum*), great brome grass (*Bromus diandrus*), wild oats (*Avena fatua*), annual ryegrass (*Lolium rigidum*) and *Brachiaria humidicola* (BNI-positive control) were grown in hydroponics, and the impact of their root exudates on NO_3^- production by *Nitrosomonas europaea* was measured in a pure-culture assay. A pot study (soil-based assay) was then conducted to confirm the ability of the weeds to inhibit nitrification in whole soils. All of the tested weeds slowed NO_3^- production by *N. europaea* in the pure-culture assay and significantly inhibited potential nitrification rates in soil-based assays. Root exudates produced by wild radish were the most inhibitory, slowing NO_3^- production by the pure culture of *N. europaea* by $53 \pm 6.1\%$ and completely inhibiting nitrification in the soil-based assay. The other weed species all had BNI capacities comparable to that of *B. humidicola* and significantly higher than that previously reported for wheat cv. Janz. This study demonstrates that several commonly occurring weed species have BNI capacity. By altering the N cycle, and retaining NH_4^+ in the soils in which they grow, these weeds may gain a competitive advantage over species (including crops) that prefer NO_3^- . Increasing our understanding of how weeds compete with crops for N may open avenues for novel weed-management strategies.

Additional keywords: ammonium, nitrification, nitrification inhibitors, weed ecology.

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Introduction

Weeds are estimated to cost the Australian agricultural, forestry and horticulture sectors ~AU\$4 billion annually through reductions in yield, reduced product quality due to contamination and the direct costs of control programs (Sinden *et al.* 2004). A similar value has been placed on the environmental, landscape and amenity costs (Sinden *et al.* 2004). Greater understanding of the traits that allow weeds to persist and outcompete crops may lead to the development of novel control methods.

Jones *et al.* (2005) found that within the southern and western cereal cropping regions of Australia, the three most economically damaging weed species were wild oats (*Avena* spp.), annual ryegrass (*Lolium rigidum*) and wild radish (*Raphanus raphanistrum*). A survey conducted in 2008 found that great brome grass (*Bromus diandrus*), although considered a minor weed, is emerging as an increasing threat to productivity in Western Australia, outranking wild oats in terms of growers' perceptions of weed importance (Hashem *et al.* 2011).

With the expansion of herbicide resistance in weeds such as wild radish, annual ryegrass and wild oats (Michael *et al.* 2010; Boutsalis *et al.* 2012), there is increasing interest in

developing a greater understanding of the traits that offer weeds a competitive advantage, with a view to developing alternative weed-management strategies.

Nitrification, the microbially mediated conversion of ammonium (NH_4^+) to nitrate (NO_3^-), is a key step in the global nitrogen (N) cycle. Ammonia oxidation ($\text{NH}_4^+ \rightarrow \text{NO}_2^-$), the first and rate-limiting step in nitrification, is of interest in agricultural systems because it is the starting point of several N-loss pathways including leaching of highly mobile NO_3^- and gaseous N loss via denitrification (Focht and Verstraete 1977).

Some weed species have been shown to affect nutrient and microbial dynamics in the soils in which they grow. For example, some grass species including *Lolium perenne*, *Bromus tectorum*, *Andropogon gayanus*, *Brachiaria humidicola* and *Hyparrhenia diplandra* slow nitrification rates in soils relative to bare soil or soils where other plant species are growing (Wheatley *et al.* 1990; Rossiter-Rachor *et al.* 2009). Wild radish is known to produce allelopathic and biofumigant compounds in its aboveground tissues and these compounds have been shown to cause shifts in the soil microbial community, including organisms that mineralise N and oxidise ammonia (Hollister

et al. 2013). Brome grass is known for its ability to compete strongly with crops for N, and at least one species of brome (*B. tectorum*) has been shown to decrease N mineralisation significantly in soils that it has invaded (Evans *et al.* 2001; Blank and Morgan 2012). It is known that nitrification rates are suppressed in the soils below mature swathes of some grass species including *A. gayanus*, *B. humidicola* and *H. diandra* (Lata *et al.* 2004; Rossiter-Rachor *et al.* 2009; Subbarao *et al.* 2009).

If weed species are actively altering the N cycle in their rhizospheres, thereby gaining a competitive advantage over crops or native plant species, it may be possible to decrease their persistence by managing the form and timing of N applications. Ishikawa *et al.* (2004) established the term 'biological nitrification inhibition' (BNI) to describe the ability of the tropical grass *B. humidicola* to suppress ammonia oxidation in soils. The authors of a series of studies on this species reported that inhibition is caused by several compounds released into the rhizosphere from plant roots (Ishikawa *et al.* 2004; Subbarao *et al.* 2006, 2007, 2008; Gopalakrishnan *et al.* 2007).

Not all plants exhibit BNI capacity. For example, O'Sullivan *et al.* (2016) showed that a common Australian wheat cultivar (Janz) did not alter N transformations in soils. It is possible that an ability to inhibit nitrification, and preserve N in the NH_4^+ form, may provide a direct competitive advantage to weeds, especially if they have a higher affinity for NH_4^+ than do other plant species that may prefer NO_3^- (Blank and Morgan 2012).

The first step in assessing this possibility was to establish whether common weed species exhibit BNI. The aim of this study was to screen a range of weed species for BNI capacity and to determine whether any BNI from their root exudates was strong enough to decrease nitrification rates in whole soil. It was hypothesised that root exudates from weed species with BNI capacity would decrease the rate of nitrification by a pure culture of the ammonia-oxidising bacterium *Nitrosomonas europaea* and that the potential nitrification rate in soil from the root-zone of those weed species would be lower than from the soil of unplanted control pots.

Materials and methods

Weed species tested

Seeds of annual ryegrass, great brome grass, wild radish, and wild oats (*Avena fatua*) were obtained from the Australian Herbicide Resistance Initiative at the University of Western Australia, Perth, Western Australia. In addition to the weeds, the BNI-producing grass *B. humidicola* (Rendle) Schweick cv. Tully (Subbarao *et al.* 2007) was used as a positive BNI control.

Pure-culture assay to test root exudates for BNI capacity

The methods used to grow the plants, collect root exudates and test the exudates for BNI capacity were described previously (O'Sullivan *et al.* 2017). Briefly, plants were germinated on white sand in 5-cm-diameter seedling pots in a glasshouse. They were then transplanted to a hydroponics system where they were grown for 4 weeks in a nutrient solution containing $\text{CO}(\text{NH}_2)_2$ (urea) 0.9 mM, KH_2PO_4 0.3 mM, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.3 mM, K_2SO_4 0.5 mM, FeEDTA 0.3 mM, MES (2-(N-morpholino)ethanesulfonic acid) 0.5 mM, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.5 mM,

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.0002 mM, H_3BO_3 0.005 mM, $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ 0.00003 mM, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.0007 mM, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ 0.0009 mM. The pH of the nutrient solution was adjusted daily with 10 N NaOH solution to maintain pH 6–7. The hydroponics system was housed in a growth cabinet with 10 h light–14 h dark cycle, 24°C daytime temperature and 15°C night time temperature, and 70% humidity.

At least three replicates of each biological treatment were grown; higher germination rates allowed for six replicates of brome grass and *B. humidicola* and five replicates of wild oats. Hydroponically grown plants are commonly used for the initial assessment of BNI because this allows access to the roots to collect exudates without risk of damaging the root system.

After 4 weeks, the plants were removed from the hydroponics system and root exudates were collected by submerging the roots in 80 mL of 1 mM NH_4Cl solution at pH 3.5, with aeration supplied by gently bubbling air through a hypodermic needle. After 24 h, the plants were removed from the exudate-collection solution, and the shoot and root weights (wet) of the plants were recorded. The solutions containing root exudate samples were freeze-dried and stored at -20°C before testing for BNI capacity. A colourimetric method using a pure culture of *N. europaea* (ATCC 19718), described by O'Sullivan *et al.* (2017), was used to compare NO_2^- production by *N. europaea* in the presence of the root exudates and in the culture alone (uninhibited control).

Nitrification rates were calculated from a linear regression of NO_2^- formation over the incubation time. Nitrification rates were converted to BNI capacity by calculating the percentage decrease in nitrification rate in the test assays relative to the uninhibited controls (Eqn 1):

$$\text{BNI capacity (\%)} = (1 - (R_{\text{sample}}/R_{\text{control}})) \times 100 \quad (1)$$

where R is nitrification rate.

Assessment of BNI in soils (soil-based assay)

In order to confirm the BNI activity of the weed species observed in the pure-culture assays, their impact on nitrification rates in a whole soil was tested. Plants were grown in rectangular root-stock pots (7 cm by 7 cm by 20 cm) filled with a dark brown sandy loam from Gingin, Western Australia (31.34°S, 115.91°E) (Table 1). The soil pH (1:5 soil:0.01 M CaCl_2 suspension) was 6.77 ± 0.03 . This soil was chosen because it was shown in previous studies to have a relatively repeatable, moderate potential nitrification rate under the conditions of the assay ($\sim 1 \text{ mg NO}_3^- \text{ formed kg}^{-1}$

Table 1. Selected physical and chemical characteristics of the soil from Gingin (south-west Western Australia) used in pot experiments

Characteristic	Gingin sandy loam (mean \pm s.d.; $n = 3$)
Silt + clay (%)	1.56 \pm 0.3
pH(CaCl_2)	6.77 \pm 0.03
Organic carbon (%)	0.28 \pm 0.02
Mineral N ($\text{NH}_4\text{-N} + \text{NO}_3\text{-N}$) (mg kg^{-1})	9 \pm 1
Colwell P (mg kg^{-1})	6 \pm 1
Colwell K (mg kg^{-1})	31 \pm 2
KCl-40 S (mg kg^{-1})	26 \pm 1

dry soil h^{-1}) and it proved successful for detecting BNI in wheat landraces (O'Sullivan *et al.* 2016). Unplanted control pots were included to give a measure of the nitrification rates in the soil under the experimental conditions. Owing to variable germination rates, at least three replicates (wild radish) and at most six replicates (unplanted, great brome grass) were grown for each treatment.

Plants were grown for 4–5 weeks in an evaporatively cooled glasshouse set at a temperature regime of 20°C day–10°C night ($\pm 2^\circ\text{C}$) with a natural photoperiod (minimum daylength ~ 10 h in June, maximum daylength ~ 13.5 h in early November), until the grasses were approaching tillering stage. This allowed the roots to explore the full volume of the pots. After the plants reached the 2-leaf stage (~ 1 –2 weeks), the pots were fertilised weekly with 30 mL of a 10 \times concentration of the nutrient solution used in the hydroponics systems, described above. This ensured that neither the plants nor the nitrifying bacterial communities were limited by N availability in the soil.

After 4–5 weeks, soil samples were collected by removing the plants from the pots and gently shaking the soil off the roots into plastic bags. Therefore, the samples were a combination of rhizosphere and bulk soil.

The BNI capacity in whole soils was assessed by using the shaken-slurry potential nitrification rate (PNR) test as described previously (Hart *et al.* 1994). Briefly, 15 g soil was placed in 100 mL medium containing 1 mM PO_4^{3-} and 1.5 mM NH_4^+ (the PNR medium) and incubated at 26°C in a shaking incubator rotating at 100 rpm. Samples of the slurry were collected at 2, 4, 20, 24 and 48 h after the incubation was started, and the liquid fraction was analysed for NO_3^- concentration using an AA1 continuous flow analyser (SEAL Analytical, Mequon, WI, USA). The PNR was determined by completing a linear regression of NO_3^- generation over the incubation time as described by Hart *et al.* (1994).

The BNI capacity for each species was calculated by comparing the nitrification rate in the presence of the plant with the nitrification rate in the unplanted controls according to Eqn 1.

Statistical analyses

All statistical analyses were carried out using the GENSTAT Release 13.1 (VSN International, Hemel Hempstead, UK). Nitrification rates were determined by using linear regressions to calculate the slope of the curve for NO_3^- generation over time. For both the pure-culture assay and the soil-based assay, statistical differences were assessed by using multiple linear regressions with groups. Data were grouped by treatment (weed species) and the uninhibited treatment was set as the control. The response variate was NO_3^- concentration and the explanatory variate was time. Slopes (nitrification rates) were considered significantly different where $P < 0.05$. BNI capacity was calculated using Eqn 1 (above) following the statistical analysis.

Results

Impact of root exudates on nitrification rates of *N. europaea* culture (pure-culture assay)

The nitrification rate of the uninhibited, pure culture of *N. europaea* was $1.4 \pm 0.04 \text{ mg NO}_3^- \text{ L}^{-1} \text{ h}^{-1}$. In the pure-culture

assay, nitrification rates were significantly ($P < 0.05$) slower in the presence of root exudates from all weed species tested relative to the uninhibited control. Nitrification rates ranged from $0.65 \pm 0.09 \text{ mg NO}_3^- \text{ L}^{-1} \text{ h}^{-1}$ in the presence of wild radish exudates to $1.3 \pm 0.05 \text{ mg NO}_3^- \text{ L}^{-1} \text{ h}^{-1}$ in the presence of wild oats exudate (Fig. 1).

The BNI capacity, calculated based on the reduction in nitrification rates of *N. europaea* in the presence of the root exudates, varied between species (Fig. 2). Exudates from brome grass, annual ryegrass and wild radish caused higher levels of inhibition than *B. humicicola*, whereas BNI capacity in wild oats was similar to that of *B. humicicola*. Root exudates from wild radish were very strongly inhibitory, causing $>50\%$ reduction in nitrification by *N. europaea* compared with the uninhibited control. Root exudates from the grasses caused lower levels of nitrification inhibition, with BNI capacities of 25% (brome grass), 13% (annual ryegrass) and 8% (wild oats) (Fig. 2).

Impact of weeds on potential nitrification rates in whole soils (soil-based assay)

The ability of the weed species to inhibit nitrification was confirmed in the soil-based assay. The PNR of the unplanted soil was $0.43 \pm 0.07 \text{ mg NO}_3^- \text{ kg}^{-1} \text{ dry soil h}^{-1}$. Similar to the pure-culture assay, all of the weeds except wild oats significantly decreased nitrification rates in soils relative to the unplanted controls, with PNRs ranging from $0.013 \pm 0.02 \text{ mg NO}_3^- \text{ kg}^{-1} \text{ dry soil h}^{-1}$ for wild radish, to $0.41 \pm 0.1 \text{ mg NO}_3^- \text{ kg}^{-1} \text{ dry soil h}^{-1}$ for wild oats (Fig. 3, $P < 0.05$). The BNI capacities of brome grass and ryegrass were comparable to the BNI capacity of *B. humicicola*, whereas that of wild radish was very high, approaching 100% inhibition of NO_3^- formation (Fig. 4).

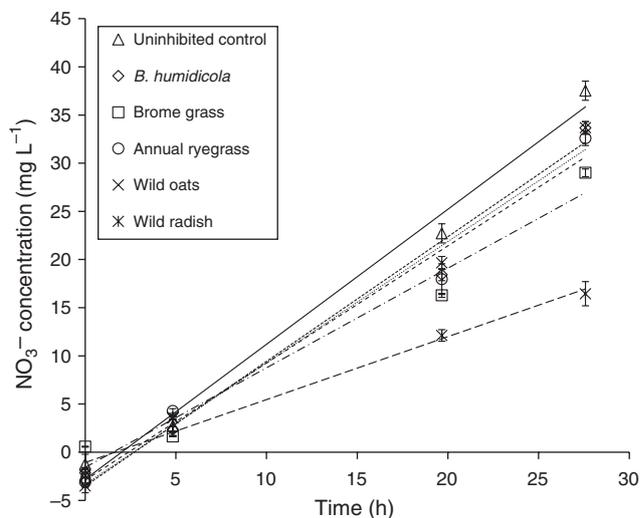


Fig. 1. Nitrification rates by a pure culture of *Nitrosomonas europaea* with and without root exudates collected from a range of weed species grown hydroponically (pure-culture assay). Error bars indicate the 95% confidence interval calculated from a minimum of three biological replicates. All root exudates caused a statistically significant reduction in NO_3^- production rate ($P < 0.05$).

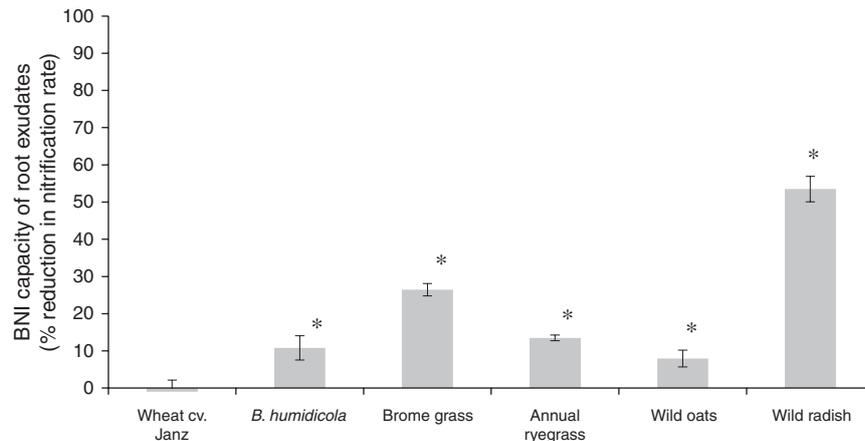


Fig. 2. Biological nitrification inhibition (BNI) capacities of weed species, calculated based on the reduction in nitrification rates by *Nitrosomonas europaea* in the presence of root exudates collected from a range of weed species grown hydroponically compared with *N. europaea* alone (control) (pure-culture assay). Error bars indicate the 95% confidence interval calculated from a minimum of three biological replicates. * $P < 0.05$ for reduction in rate relative to the unplanted control. Wheat cv. Janz from (O'Sullivan *et al.* 2016)

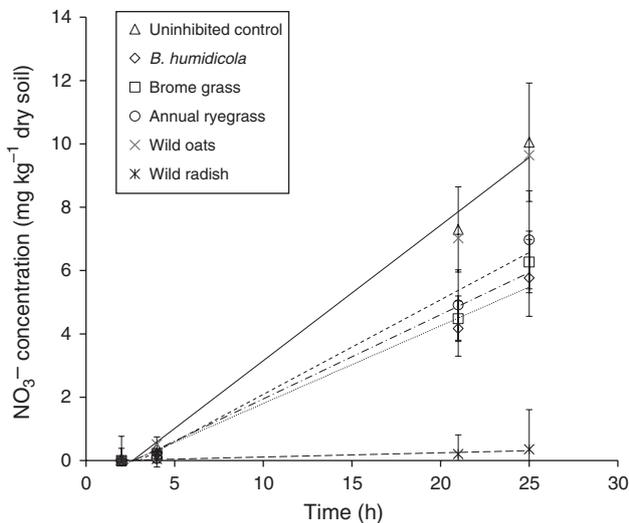


Fig. 3. Potential nitrification rates measured in pots planted with individual weed species compared with unplanted control, in soil-based assays. Error bars indicate the 95% confidence interval calculated from a minimum of three biological replicates. All species except wild oats caused a statistically significant reduction ($P < 0.05$) in NO_3^- production rate.

Discussion

Impact of root exudates from weed species on nitrification rates of *N. europaea*

Our findings provide evidence that root exudates from great brome grass, wild oats, annual ryegrass and wild radish cause significant BNI. All four species produced root exudates that caused suppression of nitrification rates of a pure culture of *N. europaea*, with wild radish clearly having the strongest effect. The BNI capacity of the three grass species tested was of the same order of magnitude as that of *B. humicicola*, which

is commonly used as a positive control for BNI capacity (Subbarao *et al.* 2009). The results of the soil-based assay confirmed the findings of the pure-culture assay. The nitrification rates in soils from each of the weed species were suppressed relative to the unplanted controls, and their BNI capacities were considerably higher than previously reported for wheat cv. Janz (O'Sullivan *et al.* 2016).

The BNI capacities measured in the soil-based assays were higher than those measured in the pure-culture assays. This is likely due to dilution effects that occurred during the collection and preparation of the root exudates for the pure-culture assay. Root exudates were collected from the hydroponically grown plants in a fixed volume of collection solution, selected to ensure that the root ball of each plant was fully submerged. After collection, the solution was freeze-dried and then rehydrated immediately before adding *N. europaea* for the pure-culture assay. The volumes of liquid used for each of these steps were much greater than volume of water in the soil-based assay. Therefore, the relative concentration of BNI compounds in the pure-culture assay was probably significantly lower than in the rhizosphere of the plants during the soil-based assay. In addition, the concentration of *N. europaea* cells was likely to have been much greater in the pure-culture assay than the soil-based assay.

Nonetheless, there was a good correlation between BNI capacities measured in the pure-culture assay and the soils-based assay. This confirms that using pure cultures to assay BNI levels in screening tests provided results that were representative of the nitrification-inhibiting capacity in a complex microbial community such as in the soil. The variability of the BNI capacity was higher in soil-based assay than in the pure culture assay, which is reflective of the more complex microbial communities and heterogeneous nature of whole soils.

Each of the methods highlighted slightly different aspects of the BNI function. The pure-culture assay showed that the exudates released by the plants were responsible for nitrification

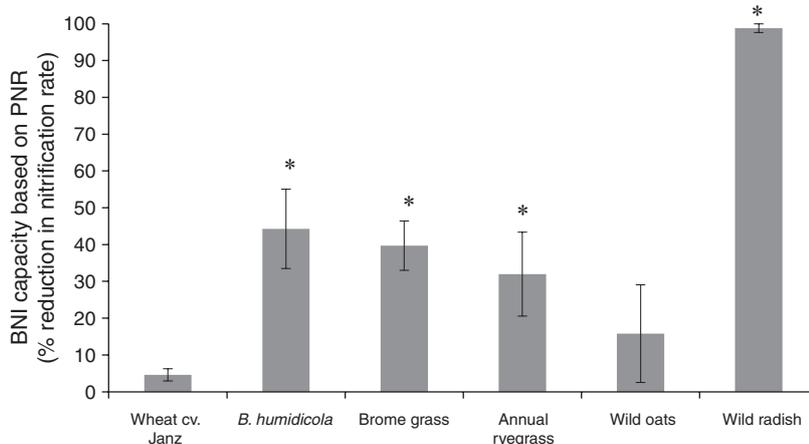


Fig. 4. Biological nitrification inhibition (BNI) capacities calculated for each species based on the potential nitrification rates (PNR), measured in pots planted with individual weed species compared with unplanted control, in soil-based assays. Error bars indicate the 95% confidence interval for each treatment from a minimum of three biological replicates. * $P < 0.05$ for reduction in rate relative to the unplanted control. Wheat cv. Janz from (O'Sullivan *et al.* 2016).

inhibition, rather than other factors such as competition between the plant and bacteria for N substrate. The soil-based assay showed that the plants released sufficient amounts of root exudates to significantly inhibit nitrification by a complex, mixed microbial community, and that the active compounds were not deactivated through interaction with soil particles or decomposition by other soil microbes.

In this set of experiments, only one cultivar of each weed species was tested, so the extent of genotypic variation for BNI capacity within each species has not been explored here. Investigations of BNI in other species have noted relatively high levels of variability in BNI capacity among genotypes (Lata *et al.* 2004; Subbarao *et al.* 2007; Tanaka *et al.* 2010; O'Sullivan *et al.* 2016). Although we provide evidence for the presence of BNI in four weed species, a broader screening of genotypes within each species would be useful to explore the level of variability for this trait.

In addition to variability for BNI capacity within species, the active compounds that cause BNI may be different between species. Subbarao *et al.* (2009) identified a cyclic diterpene as the strongest BNI compound in *B. humudicola*. This compound, which the authors named brachialactone, was shown to contribute 60–90% of the inhibitory activity released by *B. humudicola*. Plants are known to produce a wide variety of secondary metabolites for various reasons including defence and signalling (Siqueira *et al.* 1991; Bennett and Wallsgrave 1994; Theis and Lerda 2003). Several studies have shown that terpenoids released from the breakdown of aboveground biomass from several plant species are also inhibitory to nitrification (White 1994; Ward *et al.* 1997; Dietz *et al.* 2013). Along with terpenoids, known allelopathic compounds that are released by wild oats (*p*-coumaric acid and ferulic acid) (Iannucci *et al.* 2013) have been shown to be among the BNI compounds released by *B. humudicola* (Gopalakrishnan *et al.* 2007). Great brome grass and annual ryegrass may be producing compounds similar to those released by *B. humudicola*; however, more investigation is needed to explore this possibility.

The BNI capacity of wild radish, on the other hand, may be related to the production of glucosinolate (GSL) breakdown products. It is known that wild radish produces GSL and that several GSL breakdown products inhibit ammonia-oxidising microorganisms (Bending and Lincoln 2000; Brown and Morra 2009; Snyder *et al.* 2010; Hollister *et al.* 2013). However, most studies of GSL breakdown products have involved aboveground biomass or brassica meals applied to soils as either green-manure or soil amendments. It is plausible that the very high levels of BNI seen in wild radish are related to the production and release of these compounds. However, further investigation is required to confirm that GSL breakdown products are released from roots of wild radish, that their volatility does not result in their loss from the soil, and that these compounds are responsible for inhibition of ammonia-oxidising bacteria in soils.

Current literature suggests that the chemistry of BNI is complex and poorly understood and that further research is needed to increase our understanding of the mechanisms of BNI.

Implications for weed management research

Our research suggests that BNI capacity could explain some of the reports of changes in N cycling under weeds. Elevated NH_4^+ and decreased NO_3^- levels have been found, along with depressed nitrification rates, under invasive *Andropogon* grasses in northern Australia (Rossiter-Rachor *et al.* 2009). The weed *Solidago canadensis* is known to inhibit nitrification and promote ammonification in areas where it has invaded, helping it to outcompete native species (Li *et al.* 2012).

Although BNI has been reported for several plant species, most of the investigations have focused on crop and pasture species (Wheatley *et al.* 1990; Subbarao *et al.* 2007; Tanaka *et al.* 2010; Zakir *et al.* 2008). The results of our study suggest that weeds common to the Western Australian wheatbelt are capable of altering nitrification rates in their rhizospheres.

This is likely to have flow-on effects on both the soil microbial community and plants.

Further research is required to understand how weeds may be benefiting from the BNI trait, and what the implications are for the management of weeds in both cropping and native ecosystems. For example, in USA grassland ecosystems, the native crested wheatgrass (*Agropyron cristatum*, *A. desertorum*) is able to suppress invasion of cheat grass weeds (*Bromus tectorum*) by inhibiting nitrification and holding N in the NH_4^+ form (Blank and Morgan 2012). Cheat grass can use both NH_4^+ and NO_3^- but has more rapid uptake kinetics for NO_3^- . It is thought that the elevated NH_4^+ and lowered NO_3^- levels beneath crested wheatgrass allow it to outcompete cheat grass for N. Blank and Morgan (2012) suggested that NH_4^+ fertilisers and synthetic nitrification inhibitors might be useful treatments for controlling cheat grass invasion. In this case, the native species appears to possess the ability to inhibit nitrification and the invasive species does not; however, it does highlight how differences in BNI capacity and N preferences could be harnessed to develop innovative management practices for invasive species.

Similarly, Teyker *et al.* (1991) showed that maize has a preference for NH_4^+ over NO_3^- , whereas redroot pigweed (*Amaranthus retroflexus*) shows preference for NO_3^- . In experiments where N fertiliser was applied as NH_4^+ with a nitrification inhibitor, maize outcompeted redroot pigweed. The authors suggested that enhancing the proportion of N as NH_4^+ may provide more effective weed control of NH_4^+ -sensitive plants, decrease N loss from the crop root-zone, and decrease leaching of N into groundwater. If BNI is associated with NH_4^+ preference in weeds, then restriction of reduced N fertilisers (urea, NH_4^+) during weed management may help to decrease the competitive advantage of BNI-capable species.

These studies show that, by understanding how different plant species influence N transformations in their rhizospheres, we can develop strategies that favour growth of one species over another. Further research is needed to explore whether it is possible to enhance weed-control strategies by manipulating the form and/or timing of N supplied to crops.

Another aspect of the BNI phenomenon that requires further research is whether it operates at agriculturally relevant timescales. For BNI to have a meaningful impact in agricultural systems, either negative (by allowing weeds a competitive advantage) or positive (by improving N-use efficiency in BNI-capable crops), the effect would need to last over several months or several growing seasons. Some data in the literature suggest that the BNI effect could alter the N cycle in soils under invasive species in the long term. Researchers studying the invasion of northern Australian grasslands by Gamba grass (*A. gayanus*) have observed that soils that were dominated by NO_3^- before invasion became dominated by NH_4^+ after invasion (Rossiter-Rachor *et al.* 2009). This could lead to a cascade of effects on plants that preferentially take up NO_3^- over NH_4^+ , and on the soil microbial community as a whole. Several other papers make comment on the longevity of the BNI effect in crop species and the impact that this had on following crops (Moreta *et al.* 2014; Karwat *et al.* 2015). Although these studies suggest that BNI can have an impact over several cropping rotations, more research is needed to establish the temporal scale of the BNI

effect and what this means for N cycling over agriculturally relevant time scales.

Conclusions

This study showed that four weed species common to the western and southern Australian cropping zones are capable of producing root exudates that inhibit nitrification, whereas a common wheat cultivar did not possess this trait. The ability of these species to suppress nitrification in whole soils was confirmed. This highlights that increasing our understanding of how plants influence soil microbiota and associated nutrient cycling could open the door to potential novel weed-management strategies.

Conflicts of interest

The authors declare no conflicts of interest.

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