Plant influence on nitrification

Marcin W. Skiba*,†, Timothy S. George*, Elizabeth M. Bags† and Tim J. Daniell*
*Environment Plant Interactions Programme, Scottish Crop Research Institute (SCRI), Dundee DD2 5DA, U.K. and †Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen AB24 3UU, U.K.

Abstract
Modern agriculture has promoted the development of high-nitrification systems that are susceptible to major losses of nitrogen through leaching of nitrate and gaseous emissions of nitrogen oxide (NO and N\textsubscript{2}O), contributing to global warming and depletion of the ozone layer. Leakage of nitrogen from agricultural systems forces increased use of nitrogen fertilizers and causes water pollution and elevated costs of food production. Possible strategies for prevention of these processes involve various agricultural management approaches and use of synthetic inhibitors. Growing plants capable of producing nitrification suppressors could become a potentially superior method of controlling nitrification in the soil. There is a need to investigate the phenomenon of biological nitrification inhibition in arable crop species.

Introduction
Ammonium oxidation is the first and rate-limiting step in the nitrogen cycle. The contribution of nitrification to nitrogen turnover in soil varies in different environments. It is thought to play a relatively minor role in many mature climax ecosystems due to the low availability of ammonium and/or low pH that limits ammonium oxidation [1,2], but agricultural soils have very high nitrification rates [3].

The advance of modern farming systems in the 20th century introduced several changes that have caused accelerated nitrification in the soil. Intensive high-yield agriculture is dependent on modern crop varieties that require large inputs of inorganic nitrogen and intensive cultivation of soil. The shift from traditional rotation systems, increased soil tillage and drainage all contribute to the development of high-nitrification systems.

Rapid conversion of ammonium results in the creation of nitrate that is much more susceptible to increased loss from soil. The cation ammonium is bound by electrostatic forces to negatively charged clay particles and functional groups of soil organic matter [4]. This binding immobilizes ammonium and thus prevents loss from the soil by leaching. In contrast, binding of negatively charged nitrate ions to clay surfaces is much weaker. They are therefore more mobile, tend to remain in solution and are therefore liable to leakage from the rooting zone to groundwater, surface runoff and fluvial losses [5]. Moreover, under anaerobic or partially anaerobic conditions, nitrate is reduced to gaseous forms of nitrogen through denitrification. Nitric oxide, nitrous oxide and dinitrogen produced by denitrifying bacteria not only contribute to further losses of nitrogen from the soil, but nitrogen oxides are also potent greenhouse gases with large global warming potential. Nitrous oxide is also emitted directly from the plant canopy as a result of uptake of nitrogen in the form of nitrate [6]. Release of N\textsubscript{2}O from agricultural systems contributes to 70% of its global emission to the atmosphere [7].

Nitrification in agriculture
High productivity of modern agriculture relies on the industrial fixation of nitrogen, via the Haber–Bosch process, for fertilizer production. The production of nitrogen fertilizers has increased greatly in the last century. The present use of nitrogen fertilizers is likely to triple by 2050 from the current 100 Tg/year [8,9]. Approx. 90% of nitrogen fertilizer input is applied in the form of NH\textsubscript{4}+ leading to stimulation of nitrifier activity and consequently, the development of soil systems reliant on highly nitrifying conditions. In such agricultural systems, ammonium is converted into NO\textsubscript{3}– by nitrifying bacteria within days or weeks of application.

The conventional agriculture system involves cultivation of soil for weed control and seed sowing. These soil preparation procedures create fallow periods where there is no vegetation to take up accumulated nitrate that is released post-harvest and provide conditions for greater drainage. The accumulation of nitrate in soil to concentrations exceeding plant uptake requirements followed by a fallow periods are two fundamental factors determining the amount of NO\textsubscript{3}– leached from the soil [10]. This is of major environmental concern because it leads not only to losses of nitrogen from the soil, but also to contamination of ground and surface water.

There is therefore a need to synchronize nitrogen applications with plant requirements. Several strategies for optimizing nitrogen use in agricultural systems have been developed. These involve split application, deep placement of fertilizer, point injection and foliar application of urea. Such nitrogen management systems enhance the use efficiency of fertilizer by facilitating rapid nitrogen uptake by plants and reducing the residence time of nitrogen in soil which helps
competing for soil inorganic nitrogen than nitrifying bacteria, Other studies have concluded that plant roots are better in systems have been studied extensively, but the results are inconclusive [28]. In some studies, plant roots have been considered the weakest competitors, with ammonium affinity much less than that of both nitrifying and heterotrophic bacteria. This view is supported by the observation that in soils with excess available carbon, heterotrophic bacteria are able to completely immobilize all available NH4+.

The assimilation of NH4+ by plants is more efficient because it requires 4-fold less energy than the uptake of NO3− [26]. Inhibition of nitrification by plants can be viewed as a means of competition between plant roots and soil microorganisms [27]. The competition for ammonium in the soil system has been studied extensively, but the results are inconclusive [28]. In some studies, plant roots have been considered the weakest competitors, with ammonium affinity much less than that of both nitrifying and heterotrophic bacteria. This view is supported by the observation that in soils with excess available carbon, heterotrophic bacteria are able to completely immobilize all available NH4+.

Other studies have concluded that plant roots are better in competing for soil inorganic nitrogen than nitrifying bacteria, but lose out in competition with heterotrophic bacteria [29]. Therefore the plants are able to take up the nitrogen from soil that is in excess to the needs of heterotrophic microorganisms. Moreover, plants are able to take advantage of high nitrogen fluxes in the soil resulting from rapid microbial turnover. Because of the long lifespan of plants relative to micro-organisms, plants can accumulate nitrogen for growth even if they are relatively unsuccessful competitors during individual competition events.

It is commonly accepted that the growth of heterotrophic bacteria is carbon-, rather than nitrogen-, limited. However, this generalization is true only to substrates with specific carbon/nitrogen ratios. Heterotrophic bacteria and fungi have carbon/nitrogen ratios ranging from 4:1 to 12:1, but they respire approx. 50% of their carbon uptake. Consequently, there is a theoretical substrate carbon/nitrogen ratio of approx. 30:1 above which heterotrophic organisms are carbon-limited and below which they are nitrogen-limited [30]. When these organisms are limited by the nitrogen content of organic matter, they may use exogenous source of nitrogen such as soil ammonium and nitrate. Carbon input from root exudation provides a source of energy and leads to the proliferation of bacteria in the rhizosphere resulting in immobilization of the majority of the available NH4+. Limited accessibility of the substrate for AOB (ammonium-oxidizing bacteria) may be a partial explanation for low nitrification rates in mature ecosystems although the typically acidic pH in these systems may also limit bacterial nitrification [1]. The amount and type of carbon entering the soil system is strongly dependent on both plant species and environmental conditions; therefore differences observed in nitrification rates between different plants and ecosystems are not surprising.

The interaction between plants and nitrifiers is yet more complex. Early studies suggested that ammonium oxidizers are relatively minor sinks of ammonium in soil and their use of NH4+ is minimal [31]. However, recently it has been demonstrated that nitrifiers may use substantial amounts of nitrogen and that competition between plant roots and AOB exists with plants being the stronger competitor [32]. This can be related to a greater capacity of plant roots to exploit soil N-stocks. AOB are relatively limited in their capability to utilize NH4+ because of their reduced mobility. Consequently, significant microbial nitrification only occurs in soil when ammonium is present in non-limiting amounts or ammonium is localized to highly aerobic niches. Therefore reduced nitrification and reduced abundance of nitrifying bacteria in natural soils may be explained by depletion of the soil ammonium pool caused by both rapid immobilization by heterotrophic bacteria and the superior ability of plants to take up ammonium ions. Because the nitrate uptake pathway consumes more energy than the uptake of ammonium, it is possible that the ability of plants to suppress nitrification in soil evolved as mechanism to improve nutrient uptake efficiency.

Some studies have found low rates of nitrification in soils with a relatively large ammonium concentration suggesting
that there might be an additional mechanism limiting the activity of AOB. The exudation of nitrification suppressing compounds by plants has been proposed as an alternative explanation for low nitrification rates in mature environments [33].

The ability of certain plant species to release organic compounds from roots that have inhibitory effect on the activity of nitrifying bacteria has been termed BNI (biological nitrification inhibition) [34,35]. This phenomenon is known to reduce nitrification in soils of mature grassland and climax forest ecosystems [36,37]. Only recently has direct evidence for the ability of some plant species to suppress nitrification has been provided under both laboratory and field conditions. The development of a reliable method for detecting BNI activity in root exudates using a recombinant fluorescent Nitrosomonas europaea assay [38,39] has allowed relatively high-throughput screening of plant types leading to the discovery of nitrification inhibition ability in a number of arable plants [21,29].

One of the best-known BNI-capable species is Brachiaria humidicola, a tropical pasture grass adapted to acid infertile soils and humid environments, which has been extensively studied [34,40]. This work includes studies targeting the determination of the mechanisms behind BNI. Using bioassay-guided fractionation, some of the active exudates involved in nitrification inhibition have been recognized. Identified inhibitory compounds include non-esterified fatty acids, their methyl esters and a cyclic diterpene brachialoctone [14,41,42]. Their similar mode of action has also been established. These substances block nitrification activity in N. europaea by inhibiting the enzymes catalysing both reactions of ammonium oxidation via AMO and HAO (hydroxylamine oxidoreductase). The reducing power generated from the oxidation of hydroxylamine by HAO passes through cytochrome c-554 to both cytochrome aa3 oxidase and ubiquinone, which is subsequently used for the reduction of NAD(P)H as well as for the maintenance of the AMO reaction. It is thought that brachialoctalone disrupts the generation of reductive power, NAD(P)H2, by direct interference with the electron transfer pathways of the cytochrome chain in the inner membrane of Nitrosomonas [34], but multiple active compounds may employ diverse inhibitory actions to provide a sustained suppressive effect across various environments and distinct communities of ammonium oxidizers. The production and release of inhibitory compounds by B. humidicola roots is not constitutive, but is triggered by high concentration of NH4+ in the soil solution and acidic pH although the exact mechanisms activating the synthesis of BNI compounds remain unknown.

Concluding remarks

The processes occurring on the root/soil interface in relation to the interaction between the plant and soil microbial populations are both complex and poorly understood. This is particularly true for nitrifying micro-organisms that do not rely on the plant for carbon. Clearly, the rhizosphere in general and nitrifying microbiology in particular require further investigation with more direct measures needed to establish the significance of BNI to agriculture.

Plant-derived nitrification inhibitors may provide an alternative solution to allow the manipulation of nitrification in the soil [43]. In situ production of nitrification inhibitors by roots has an appeal as a low-cost alternative to SNIs. Unlike SNIs, nitrification inhibitors are stable in the soil and their production is localized to the root zone and therefore their action may be more efficient. The production of BNI by crop species under field conditions and their effectiveness in reducing nitrogen losses remains to be demonstrated, but there is the potential to mitigate nitrogen leakage and improve fertilizer use efficiency. Controlling ammonium oxidation in soil through suppression may minimize losses of nitrogen by leaching and denitrification and stimulate nitrogen flow through ammonium assimilation pathways.

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References
