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SHORT COMMUNICATION

Bacteria on leaves: a previously unrecognised source of N_2O in grazed pastures

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Nitrous oxide (N_2O) emissions from grazed pastures are a product of microbial transformations of nitrogen and the prevailing view is that these only occur in the soil. Here we show this is not the case. We have found ammonia-oxidising bacteria (AOB) are present on plant leaves where they produce N_2O just as in soil. AOB (*Nitrosospira* sp. predominantly) on the pasture grass *Lolium perenne* converted 0.02–0.42% (mean 0.12%) of the oxidised ammonia to N_2O. As we have found AOB to be ubiquitous on grasses sampled from urine patches, we propose a 'plant' source of N_2O may be a feature of grazed grassland.
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In terms of climate forcing, nitrous oxide (N_2O) is the third most important greenhouse gas (Blunden and Arndt, 2013). Agriculture is the largest source of anthropogenic N_2O (Reay *et al.*, 2012) with about 20% of agricultural emissions coming from grassland grazed by animals (Oenema *et al.*, 2005).

Grazed grassland is a major source of N_2O because grazers harvest nitrogen (N) from plants across a wide area but recycle it back onto the pasture, largely as urine, in patches of very high N concentration. The N in urine patches is often in excess of what can be used by plants resulting in losses through leaching as nitrate, as N_2O and through volatilisation as ammonia (NH_3) creating a high NH_3 environment in the soil and plant canopy; an important point that we will return to later. The established wisdom is that N_2O is generated exclusively by soil-based microbes such as ammonia-oxidising bacteria (AOB). This soil biology is represented in models designed to simulate N_2O emissions and the soil is a target for mitigation strategies such as the use of nitrification inhibitors.

We have previously shown that pasture plants can emit N_2O largely through acting as a conduit for emissions generated in the soil, which are themselves controlled to some degree by the plant (Bowatte *et al.*, 2014). In this case the origin of the emission is still the soil microbes. However, AOB have been found on the leaves of plants, for example, Norway spruce (Papen *et al.*, 2002; Teuber *et al.*, 2007) and weeds in rice paddies (Bowatte *et al.*, 2006), prompting us to ask whether AOB might be present on the leaves of pasture species and contribute to N_2O emissions as they do in soil.

We looked for AOB on plants in situations where NH_3 concentrations were likely to be high, choosing plants from urine patches in grazed pastures and plants from pastures surrounding a urea fertiliser manufacturing plant. DNA was extracted from the leaves (including both the surface and apoplast) and the presence of AOB tested using PCR. AOB were present in all the species we examined—the grasses *Lolium perenne*, *Dactylis glomerata*, *Anthoxanthum odoratum*, *Poa pratensis*, *Bromus wildenowii* and legumes *Trifolium repens* and *T. subterraneum*.

To measure whether leaf AOB produce N_2O, we used intact plants of ryegrass (*L. perenne*) lifted as cores from a paddock that had been recently grazed by adult sheep. The cores were installed in a chamber system designed to allow sampling of above- and belowground environments separately (Bowatte *et al.*, 2014). N_2O emissions were measured from untreated (control) plants and from plants where NH_3 was added to the aboveground chamber and leaves were either untreated or sterilised by wiping twice with paper towels soaked in 1% hypoclorite (Sturz *et al.*, 1997) and then with sterile water. We tested for the presence and abundance of AOB on the leaves by extracting DNA and using PCR and real-time PCR targeting the ammonia monoxygenase A (*amoA*) gene, which is characteristic of AOB. AOB identity was established using cloning and DNA sequencing. Further details of these experiments can be found in the Supplementary Information.

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The addition of NH_3 to untreated plants significantly stimulated N_2O emissions ($P<0.001$) compared with the controls; by contrast, the plants with sterilised leaves produced significantly less N_2O than controls ($P<0.001$) even with NH_3 added (Figure 1) providing strong evidence for emissions being associated with bacteria on the leaves. Control plants did emit N_2O suggesting there was either sufficient NH_3 available for bacterially generated emissions and/or other plant-based mechanisms were involved (Bowatte *et al.*, 2014).

The major AOB species identified was *Nitrosospira* strain III7 that has been previously shown to produce N_2O (Jiang and Bakken, 1999). We measured 10^9 AOB cells per m^2 ryegrass leaf, assuming a specific leaf area of 250 $cm^2\,g^{-1}$ leaf.

The rate of production of N_2O (0.1–0.17 mg N_2O-N per m^2 leaf area per hour) can be translated to a field situation using the leaf area index (LAI)—1 m^2 leaf per m^2 ground would be an LAI of 1. LAI in a pasture can vary from <1 to >6 depending on the management (for example, Orr *et al.*, 1988). At LAI of 1, the AOB leaf emission rate would equate to a N_2O emission rate of about 0.1–0.3 mg N_2O-N per m^2 ground per hour. By comparison, the emission rates measured after dairy cattle urine (650 kg N ha^{-1}) was applied to freely and poorly drained soil were 0.024–1.55 and 0.048–3.33 mg N_2O-N per m^2 ground per hour, respectively (Li and Kelliher, 2005).

The fraction of the NH_3 that was converted to N_2O by the leaf AOB was 0.02–0.42% (mean 0.12%). The mean value is close to that measured for *Nitrosospira* strains including strain III7 isolated from acidic, loamy and sandy soils where values ranged from 0.07 to 0.10% (Jiang and Bakken, 1999). This is good evidence that the AOB on leaves have the capacity to produce N_2O at the same rate as AOB in soils. We do not suggest that leaf AOB will produce as much N_2O as soil microbes; however, because leaf AOB have access to a source of substrate—volatilised NH_3—that is unavailable to soil microbes and may constitute 26% (Laubach *et al.*, 2013) to 40% (Carran *et al.*, 1982) of the N deposited in the urine, N_2O emissions from these aboveground AOB are additional to soil emissions. Further research is required to identify the situations in which leaf AOB contribute to total emissions and to quantify this contribution.

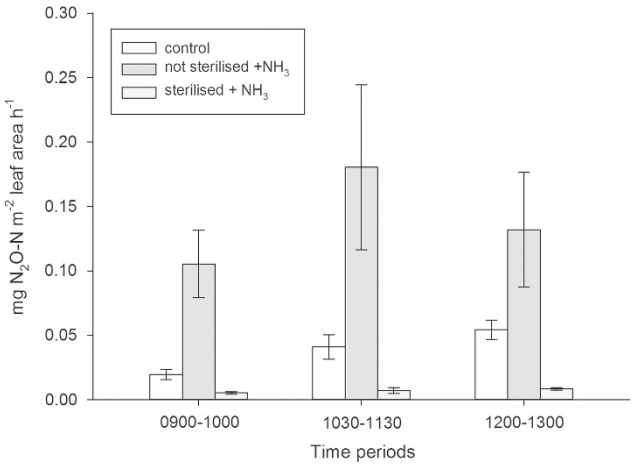

Figure 1 Effect of an elevated NH_3 atmosphere and surface sterilisation of leaves on leaf N_2O emissions measured over 1-h periods on three occasions during the day. Values are means (s.e.m.), where $n=7$.

Conflict of Interest

The authors declare no conflict of interest.

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References

Blunden J, Arndt DS. (2013). State of the climate in 2012. *B Am Meteorol Soc* **94**: S1–S258.

Bowatte S, Ishihara R, Asakawa S, Kimura M. (2006). Characterization of ammonia oxidizing bacteria associated with weeds in a Japanese paddy field using amoA gene fragments. *Soil Sci Plant Nutr* **52**: 593–600.

Bowatte S, Newton PCD, Theobald P, Brock S, Hunt C, Lieffering M *et al.* (2014). Emissions of nitrous oxide from the leaves of grasses. *Plant Soil* **374**: 275–283.

Carran RA, Ball PR, Theobald PW, Collins MEG. (1982). Soil nitrogen balances in urine-affected areas under two moisture regimes in Southland. *N Z J Exper Agric* **10**: 377–381.

Jiang QQ, Bakken LR. (1999). Nitrous oxide production and methane oxidation by different ammonia- oxidizing bacteria. *Appl Environ Microbiol* **65**: 2679–2684.

Laubach J, Taghizadeh-Toosi A, Gibbs SJ, Sherlock RR, Kelliher FM, Grover SPP. (2013). Ammonia emissions from cattle urine and dung excreted on pasture. *Biogeosciences* **10**: 327–338.

Li Z, Kelliher FM. (2005). Determining nitrous oxide emissions from subsurface measurements in grazed pasture: A field trial of alternative technology. *Aust J Soil Res* **43**: 677–687.

Oenema O, Wrage N, Velthof GL, Van Groenigen JW, Dolfing J, Kuikman PJ. (2005). Trends in global nitrous oxide emissions from animal production systems. *Nutr Cycl Agroecosys* **72**: 51–65.

Orr RJ, Parsons AJ, Treacher TT, Penning PD. (1988). Seasonal patterns of grass production under cutting or continuous stocking managements. *Grass Forage Science* **43**: 199–207.

Papen H, Geβler A, Zumbusch E, Rennenberg H. (2002). Chemolithoautotrophic nitrifiers in the phyllosphere of a spruce ecosystem receiving high atmospheric nitrogen input. *Curr Microbiol* **44**: 56–60.

Reay DS, Davidson EA, Smith KA, Smith P, Melillo JM, Dentener F *et al*. (2012). Global agriculture and nitrous oxide emissions. *Nat Clim Change* **2**: 410–416.

Sturz AV, Christie BR, Matheson BG, Nowak J. (1997). Biodiversity of endophytic bacteria which colonize red clover nodules, roots, stems and foliage and their influence on host growth. *Biol Fertility Soils* **25**: 13–19.

Teuber M, Papen H, Gasche R, Eßmüller TH, Gebßler A. (2007). The apoplast of norway spruce (*Picea abies*) needles as habitat and reaction compartment for autotrophic nitrifiers. In: Sattelmacher B, Horst WJ (eds) *The Apoplast of Higher Plants: Compartment of Storage, Transport and Reactions*. Springer, pp 405–425.

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