Agricultural soils are the largest single source of anthropogenic N\textsubscript{2}O to the atmosphere, yet spatially and temporally representative data are needed to fully understand the transformation of applied N to N\textsubscript{2}O. Greater knowledge of the factors affecting variability in N\textsubscript{2}O emissions could be achieved with advanced methods for continuously tracking how N addition influences N\textsubscript{2}O fluxes. Nitrous oxide production in agricultural soil typically involves biogenic transformation of both synthetic fertilizer N and resident pools of N, where the N required for N\textsubscript{2}O production may have entered the soil via atmospheric or fluvial deposition, synthetic fertilizer application, or mineralization processes. It is difficult to assess the proportion of N\textsubscript{2}O emissions attributed directly to fertilizer N addition without using isotopic tracers, such as \textsuperscript{15}N (Panek et al., 2000; Bergsma et al., 2001; Pérez et al., 2001; Park et al., 2012). Tracking \textsuperscript{15}N-N\textsubscript{2}O in the field, however, is problematic without a method for continuous measurement because N\textsubscript{2}O emissions at the surface of soils are highly episodic and spatially variable (Parkin, 1987; Yamulki et al., 2000; Jones et al., 2011). Methods are needed to quantify the fraction of added N emitted as N\textsubscript{2}O under field conditions and the duration of this response (Kellie et al., 2002; Sagar et al., 2013).

The effect of fertilizer N addition on soil N\textsubscript{2}O emissions reportedly persists for varying lengths of time, ranging from 2 to 20 wk (Breitenbeck and Bremmer, 1986; Flessa et al., 1995; Bouwman et al., 2002; Phillips et al., 2007; Denmead et al., 2010), with fractions of fertilizer N emitted as N\textsubscript{2}O varying from 1 to 5\% (Intergovernmental Panel on Climate Change, 2006). Greater lengths of time and higher percentages of added N emitted as N\textsubscript{2}O have been reported for heavily fertilized (160 kg ha\textsuperscript{-1}) soils in tropical climates (Denmead et al., 2010), while smaller lengths of time and lower percentages of N emissions have been reported for lightly fertilized soils in semiarid climates (Barton et al., 2008; Phillips et al., 2009). However, directly linking N addition to N\textsubscript{2}O emissions at high spatiotemporal resolution in the field has been limited by technical constraints.
Investigation of when, how much, and under what conditions added N is emitted as N₂O at the surface of soil in the field would complement the growing body of studies aimed toward constraining the effects of land management on N₂O emissions (Hénault et al., 2005; Blagodatsky and Smith, 2012).

Application of ¹⁵N laboratory techniques, including isotope pairing and molecular probing, has provided insight into gaseous N removal pathways, such as nitrification, denitrification, co-denitrification, nitrifier denitrification, and anaerobic NH₄ oxidation (anammox) with measurement of N isotopes, such as ¹⁴N¹⁴N¹⁶O, ¹⁴N¹⁵N¹⁶O, ¹⁵N¹⁴N¹⁶O, ¹⁵N¹⁵N¹⁶O, ²⁸N₂, ²⁹N₂, and ³⁰N₂ using isotope ratio mass spectrometry (IRMS) (Yamulki et al., 2000; Köster et al., 2011; Long et al., 2013). Additionally, researchers have demonstrated the importance of O isotopes, such as ¹⁸O, when tracing specific biochemical pathways of N₂O (Kool et al., 2007). While IRMS can provide detailed isotopic flux profile information, high temporal resolution of fluxes in the field cannot be achieved with this technique. Field-based instruments capable of tracking how ¹⁵N-labeled N affects fluxes of ¹⁴N¹⁴N¹⁶O (±28-N₂O), ¹⁴N¹⁵N¹⁶O + ¹⁵N¹⁴N¹⁶O (±29-N₂O), and ¹⁵N¹⁵N¹⁶O (±30-N₂O) could be used to infer interactions and potential mixing between the added and resident soil N pools, such as apparent priming effects (Hart et al., 1986; Di and Cameron, 2008). (As appropriate, we use ²⁸N₂O to mean ¹⁴N¹⁴N¹⁶O, ²⁹N₂O to indicate ¹⁴N¹⁵N¹⁶O + ¹⁵N¹⁴N¹⁶O, and ³⁰N₂O to mean ¹⁵N¹⁵N¹⁶O, where isotopes of O and site preference for ¹⁵N substitution are not resolved. We use ¹⁴N-N₂O to refer to all ¹⁴N-containing N₂O: ¹⁴N¹⁴N¹⁶O = 2 × ²⁸N₂O + ²⁹N₂O, and similarly ¹⁵N¹⁵N¹⁶O = 2 × ²⁹N₂O + ³⁰N₂O.) Apparent priming effects differ from actual priming effects in that substrate addition does not stimulate soil respiration or plant N uptake (Kuziyakov et al., 2000). In the absence of enhanced microbial or plant activities, N₂O produced through exchanges with unlabeled soil ¹⁴N and labeled soil ¹⁵N would be considered as an apparent priming effect (Jenkinson et al., 1985; Hart et al., 1986; Kuziyakov et al., 2000). This substitution between labeled and unlabeled soil N indicates that the labeled substrate is mixed enough in the soil pore spaces to facilitate ¹⁴N and ¹⁵N exchange but does not necessarily indicate stimulation or “priming” of resident soil N cycling (Hart et al., 1986; Kuziyakov et al., 2000).

The need to quantify interactions between biogenic emissions of N₂O and N fertilization under field conditions is growing as more studies quantify linkages between crop and improved pasture production and emissions of N₂O to the atmosphere (Park et al., 2012). Continuous measurement of N₂O and other greenhouse gases is most commonly achieved with automated chamber systems combined with continuous analyzers such as portable gas chromatographs (Denmead, 2008; Griffith et al., 2012) or pseudo-continuous analyzers such as portable gas chromatographs (Barton et al., 2008). Optical (spectroscopic) analyzers based on near infrared and Quantum cascade lasers (QCLs) or FTIR are becoming increasingly commercially available from manufacturers including Aerodyne Research (http://www.aerodyne.com/), Los Gatos Research (http://www.lgrinc.com/), Picarro (http://www.picarro.com/), Tiger Optics (http://www.tigeroptics.com), and Gasmet (http://www.gasmet.fi/). The FTIR system used in this study is now commercially available from Ecotech (http://www.ecotech.com.au/).

These techniques effectively measure net fluxes of species such as N₂O, where the N contributing to the N₂O measurement is derived from both resident and fertilizer N pools (Di and Cameron, 2008). In this study, we distinguished soil emissions of N₂O due to ¹⁵N addition from N₂O derived from resident soil N with a new method for quantification of ¹⁵N-N₂O isotopologues in situ using a high-precision FTIR developed at the University of Wollongong, New South Wales, Australia, configured with an automated chamber system.

Our primary goal was to determine the potential for a portable FTIR analyzer to continuously quantify N₂O isotopologues (¹⁴N¹⁴N¹⁶O, ¹⁴N¹⁵N¹⁶O, ¹⁵N¹⁴N¹⁶O, and ¹⁵N¹⁵N¹⁶O) at low levels of ¹⁵N addition through two field trials at an undisturbed grassland site during the southern hemisphere summer of 2011–2012. To assess potential priming effects associated with N addition, we needed to quantify soil respiration and ¹⁵N recovered in plant biomass as well as fluxes of CO₂ and ²⁸N₂O before and after ¹⁵N addition. Successful detection of N₂O isotopologues would allow us to quantify the fraction of added ¹⁵N emitted as N₂O and track how fluxes vary with moisture, temperature, and time under field conditions. Further, relative fluxes of ³⁰N₂O, ²⁹N₂O, and ²⁸N₂O could be used to explore potential interactions between added N and the resident soil N pool following amendment with ¹⁵N-labeled substrates, such as KNO₃ or CO(NH₂)₂.

Materials and Methods

Field Site

The study site is a 10- by 20-m area at the center of a 73-ha managed pasture located 10 km from Nowra (34°50′S, 150°40′E) along the coast of New South Wales, Australia. Nowra rainfall and minimum and maximum temperatures for the period October 2011 to January 2012 by month are given in Table 1. The pasture is seeded regularly to maintain a stand of Kikuyu grass (Pennisetum clandestinum Hochst. ex Chiov.) and perennial ryegrass (Lolium perenne L.) that are harvested for silage. The pasture is seeded regularly to maintain a stand of Kikuyu grass (Pennisetum clandestinum Hochst. ex Chiov.) and perennial ryegrass (Lolium perenne L.) that are harvested for silage.

The underlying light clay B horizon is subject to temporary waterlogging (contains traces of sesquioxide mottling and thin tubular concretions below 0.6 m). The study area is typical of the low-lying coastal floodplains of New South Wales, with an elevation of 0.5 m above sea level. The soil profile has acid sulfate material (i.e., traces of yellow jarosite mottling) below a depth of 1 m. The organic horizon is approximately 0.2 m deep (a dark silty light clay, 10YR 3/1, with a pH of 5.6 in CaCl₂). The underlying light clay B horizon is subject to temporary waterlogging (contains traces of sesquioxide mottling and thin tubular concretions below 0.6 m). The study area is a managed irrigation operation, balancing the effects of the high regional water tables and irrigation on fodder production and reducing any potential of pyritic oxidation within the profile (Indraratna et al., 2005). Irrigation nozzles from the pivot arm were located 50 to 500 m from the pivot center (in the middle of the pasture).

Table 1. Mean daily maximum and minimum temperatures and total rainfall recorded at the Nowra weather station located 10 km south of the field site for the months October 2011 to January 2012 (Bureau of Meteorology, http://www.bom.gov.au/climate/data/).

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<tr>
<td>Simulated rainfall</td>
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<td>0</td>
<td>6</td>
<td>42</td>
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<tr>
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<td>Min. temperature</td>
<td>10.8</td>
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Our study site, located adjacent to the pivot center and apart from the experimental treatments, was not irrigated or fertilized. Soil chemical properties at the site were determined at the New South Wales Diagnostic and Analytical Services laboratory (http://www.dpi.nsw.gov.au/aboutus/services/das) using accredited soil laboratory procedures (ISO/IEC 17025) on cores (four replicates, 0.25-m diameter by 0.1-m depth) collected at the site on 1 Dec. 2011. Average (SD in parentheses) electrical conductivity (1:5 in water), NH₄⁻N and NO₃⁻N (both KCl extractable), and total P (hot acid digest) were 0.52 (0.22) dS m⁻¹, 14.75 (3.86) mg kg⁻¹, 18.50 (6.35) mg kg⁻¹, and 1.70 (5.72) g kg⁻¹, respectively. Here, we define the term resident inorganic N as the inorganic soil N pool before N addition, which is estimated as the sum of NH₄⁻N and NO₃⁻N (~33 mg kg⁻¹). The term resident N refers to both organic and inorganic forms of soil N.

Field Site Instrument Setup

The instrument caravan was deployed at the center of the pasture near the irrigation pivot from September 2011 until February 2012. A weather station was located 15 m south of the instrument caravan and included a rain gauge (TE525WS-L, Texas Electronics), sonic anemometer (CSAT-3, Campbell Scientific), air temperature sensor (HMP-45, Vaisala), quantum photosynthetically active radiation sensor (LI-190, LI-COR Environmental), net radiometer (Q7.1, Campbell Scientific), soil temperature probes (T108, Campbell Scientific), and three soil moisture sensors (CS616, Campbell Scientific). Both soil temperature and soil moisture sensors were inserted 4 cm belowground horizontally, or parallel to the surface. One probe (T108) recorded air temperature 4 cm above the ground. Moisture probe output ranged from 14 μs in air to 42 μs when immersed in water. Weather and soil data were collected every minute using a datalogger (CR5000, Campbell Scientific), and 30-min-average data were synchronized to the chamber flux measurements.

Five chambers were deployed in a semicircle 10 m northeast to southeast from the instrument caravan by anchoring stainless steel bases into the soil (0.50- by 0.50- by 0.10-m depth) until they were flush with the surface. Chamber tops (0.30 m height) were screwed onto the bases following the design of Breuer et al. (2000) and as described by Denmead et al. (2010). Tops were covered with foil so that the area inside the chamber would be dark when closed. A University of Wollongong FTIR spectrometer (described below) was located inside the instrument caravan and connected to each of the five chambers using 12-m lengths of 0.64-cm (1/4-inch) o.d. Synflex Dekabon tubing and a switching manifold. Each chamber had a short length of 0.64-cm Dekabon tubing connecting to the outside to equalize pressure. Plant height inside the chambers and above the soil temperature and moisture sensors was maintained at approximately 0.02 m for the duration of the experiment by clipping the plants weekly. All plants clipped following ¹⁵N addition were processed for ¹⁵N recovery (described below).

Soil cores were collected near the chambers to determine the relationship between soil water-filled pore space (WFPS) and moisture probe data. Cores were collected every 7 to 10 d from 1 Nov. 2011 to 30 Jan. 2012. Custom, stainless-steel core barrels (0.1-m diameter by 0.06-m depth) with slightly beveled, sharpened ends were used to collect the cores. The cores were collected and immediately placed in cold storage for transport.

Wet mass and oven-dry mass (105°C) were determined for each core, and volumetric moisture was calculated using the measured bulk density. The WFPS was calculated using a particle density of 2.64 g cm⁻³. The average WFPS values for the five cores were regressed on microsecond data collected by the soil moisture sensors (three-sensor average) at the time the soil cores were collected.

The FTIR was set up to measure fluxes from each chamber every 3 h following a cycle of 30 min per chamber. During the sixth 30-min period of each 3-h cycle, samples were analyzed from two inlets at 0.5- and 1.5-m heights on a separate mast (these data were intended for an ancillary flux-gradient study and are not reported here). The chambers remained open until ready for sampling, when the FTIR pump circulated air in a closed loop from the head space of the chamber through the FTIR spectrometer sample cell and back to the chamber. During the first 6 min of each 30-min chamber period, gas concentration data were collected while the open chamber was flushed with ambient air. The lid was then closed, and gas concentrations were measured for 18 min. A final 6 min of measurements with the open chamber completed the 30-min period. The FTIR continuously determined mole fractions of CO₂, CH₄, CO, N₂O, N₂O isotopologues, and ¹³CO₂ with 1-min time resolution. Only CO₂ and N₂O data are presented here. Only measurements from 8 to 22 min during chamber closure were used to calculate fluxes.

Nitrogen-15 Labeling Procedure

We used two independent trials to evaluate emissions of N₂O following the application of ¹⁵N. For Trial 1, we applied two levels of ¹⁵N (0.4 and 0.8 g m⁻²) for the purpose of achieving detection of ¹⁵N-N₂O while minimizing potential priming effects of added N on N₂O production (Di and Cameron, 2008). Initially, it was important to identify if ¹⁵N-N₂O isotopologues could be detected by the FTIR system at 0.4 g ¹⁵N m⁻². These N levels are low with respect to agronomic applications (agronomic N addition is easily an order of magnitude greater than our N levels) and represented approximately 12 to 24% of the resident inorganic N pool. Trial 1 began 2 Dec. 2011 with collection of ambient N₂O fluxes at each chamber every 3 h until 16 Dec. 2011. On 16 Dec. 2011, ¹⁵N (99%) dissolved in deionized (DI) water was applied to four chambers and DI water was applied to one chamber (control) just before chamber closure. For two of these chambers, we applied 0.8 g m⁻² ¹⁵N as KNO₃ (99% ¹⁵N) dissolved in 0.5 L of DI water (identified as T1-N1 and T1-N2). For two additional chambers, we applied 0.4 g m⁻² ¹⁵N as CO(NH₂)₂ (99% ¹⁵N) dissolved in 0.5 L of DI water (identified as T1-U1 and T1-U2). For the remaining chamber, only 0.5 L of DI water (identified as T1-C) was applied. While two different sources of N were used, the relatively large soil background of inorganic N (~33 mg N kg⁻¹) was expected to dilute the added ¹⁵N pool, possibly mitigating any effects that the form of N may have had on the ¹⁴N recovered in soil, plant, microbial, and N₂O isotopologue pools (Morse and Bernhardt, 2013). The solutions were applied evenly to each chamber using a removable grid (5 x 5) overlay, with 25 cells evenly spaced across the surface of the chamber area. The solutions (10 mL) were applied with a syringe over the soil in each cell. After delivery into 25 cells (0.25 L), the procedure was repeated across all cells to deliver a total of 0.5 L per chamber. Within 5 min of solution application, the chambers were closed and fluxes were measured.
The same procedure was used to apply 0.5 L of DI water over the soil moisture sensors outside the chambers. Fluxes for Trial 1 were collected until 28 Dec. 2011. On 28 December, the plant and soil materials inside each chamber were destructively collected to determine $^{15}$N remaining in the soil and plant pools. The aboveground plant material was clipped to ground level, and one soil core (0.048-m diameter) was collected inside each chamber. Each core was split into two depths (0.0–0.1 and 0.1–0.2 m). Plant and soil samples were placed in cold storage (4°C) for transport to the laboratory and processing the following day. The chambers were moved on 28 December to new locations approximately 2 m south of the original chamber locations. These new chamber sites were used in Trial 2.

For Trial 2, ambient $\text{N}_2\text{O}$ fluxes were measured beginning 2 Jan. 2012 at the new chamber locations. On 17 January, $^{15}$N was applied to four chambers and DI water only to one chamber (the control) just before chamber closure. We applied 0.4 g m$^{-2}$ of $^{15}$N as KNO$_3$ (99% $^{15}$N) dissolved in 0.5 L of DI water to two chambers (identified as T2-N1 and T2-N2). We also applied 0.4 g m$^{-2}$ of $^{15}$N as CO(NH$_2$)$_2$ (99% $^{15}$N) dissolved in 0.5 L of DI water to two additional chambers (identified as T2-U1 and T2-U2). For the remaining chamber, we applied 0.5 L of DI water only (identified as T2-C). In this second trial, the dose was consistent among chambers, but we maintained consistency between trials by applying both forms of N (KNO$_3$ and CO(NH$_2$)$_2$). The grid overlay method was used again to ensure even application of solution across the chamber footprint. January was much drier than December, so rainfall was simulated with occasional manual additions of DI water as required to bring the WFPS near 40% on 18, 20, and 23 January. For each DI water addition to the chambers, an equal amount of DI water was also applied over the soil moisture sensors outside the chambers. On 30 January, plant and soil materials inside each chamber were collected for $^{15}$N analysis as described for Trial 1.

### Plant and Soil Laboratory Analyses

Soils were sieved (2 mm) to separate roots from soil. Sieved soils were further picked for roots that fell through the sieve. Roots from both soil depths were washed and, together with the aboveground biomass, dried (60°C for 48 h) and weighed. The aboveground biomass from the clippings collected between 16 and 28 December during Trial 1 and between 17 and 30 January during Trial 2 was used to estimate the aboveground plant biomass produced during these time periods.

Microbial biomass N and $^{15}$N enrichment were measured using fumigation extraction (Bruulsema and Duxbury, 1996). After thoroughly homogenizing the soil sample, a 25-g fresh subsample was added to 50 mL of 0.05 mol L$^{-1}$ K$_2$SO$_4$. Another 25-g subsample was fumigated with chloroform for 5 d in a vacuum desiccatar and then also added to 50 mL of 0.05 mol L$^{-1}$ K$_2$SO$_4$. The samples were shaken for 1 h and filtered through preleached Whatman no. 1 filter paper. Aliquots of the extracts were analyzed for total organic C (TOC) and total N on a TOC analyzer with an N measuring unit attached (Shimadzu TOC- VCPN; Shimadzu Scientific Instruments). Another aliquot of 6 mL was dried down in an oven (60°C) and analyzed for $^{13}$C on an IRMS (Delta V, Thermo Finnigan).

Microbial N was calculated as the difference between N in the fumigated and unfumigated samples divided by 0.54 (Brookes et al., 1985). We calculated the $^{15}$N atom% in the microbial biomass ($^{15}$N$_{mic}$) using

$$^{15}\text{N}_{mic} = \frac{^{15}\text{Nf} - ^{15}\text{Ne}}{^{15}\text{Nf} - ^{15}\text{Ne}}$$  \[1\]

where $^{15}$N$_{f}$ and $^{15}$N$_{e}$ are the $^{15}$N atom% and total amount of N in the fumigated extracts and $^{15}$N$_{f}$ and $^{15}$N$_{e}$ are the $^{15}$N atom% and total amount of N in the unfumigated extracts. We calculated $^{15}$N recovery in the microbial N pool in the $^{15}$N-labeled chambers ($^{15}$N$_{rec,mic}$) using

$$^{15}\text{N}_{rec,mic} = \frac{^{15}\text{N}_{mic,l} - ^{15}\text{N}_{mic,n}}{^{15}\text{N}_{label} - ^{15}\text{N}_{mic,n}}$$  \[2\]

where $^{15}$N$_{mic,l}$ and $^{15}$N$_{mic,n}$ are the total amount of N and $^{15}$N atom% in the microbial biomass labeled with $^{15}$N, $^{15}$N$_{mic}$ is the average $^{15}$N atom% in the microbial biomass not labeled with $^{15}$N, and $^{15}$N$_{label}$ is the $^{15}$N atom% of the label. We calculated $^{15}$N recovery in the plant N pools and in the total soil N pool in a similar way. We calculated total $^{15}$N recovery by summing the $^{15}$N recovery in plants and soil.

### Fourier-Transform Infrared Description and Calibration

The University of Wollongong FTIR trace gas and isotope analyzer was described fully by Griffith et al. (2012) and is only briefly described here. The analyzer consists of a mid-infrared FTIR spectrometer with 1 cm$^{-1}$ resolution (IRcube, Bruker Optik) coupled to an optical multipass sample cell with 24-m path length and 3.5-L volume. The complete analyzer is now available commercially as Spectronus, Ecotech Pty Ltd.) Sample air from the chambers was dried in a Nafion/Mg(ClO$_4$)$_2$ dryer and circulated in a closed loop through the cell and selected chamber at 1.7 standard L min$^{-1}$ with a four-stage oil-free diaphragm pump (MV2, Vacuubrand); thus, the e-folding time for gas exchange in the cell was ~2 min. A switching manifold selected the five chambers and mast inlets sequentially in a repeated 3-h cycle. Spectra were recorded from 1800 to 7500 cm$^{-1}$ continuously, averaged, and analyzed and the results logged every 1 min to provide real-time mole fractions of the target species. The analyzer and chamber switching system was fully automated and operated continuously from October 2011 to January 2012.

Each recorded spectrum was analyzed by a least squares fitting technique in several spectral regions to determine the concentrations of trace gases and isotopologues simultaneously (Griffith et al., 2012). The concentrations of individual isotopologues $^{14}$N$^{14}$N$^2$O, $^{15}$N$^{14}$N$^2$O, $^{14}$N$^{15}$N$^1$O, and $^{15}$N$^{15}$N$^2$O were independently retrieved from the spectra from fits of a customized window 2090 to 2220 cm$^{-1}$. The spectral line parameters including additional isotopologue lines required to fit the enhanced amounts for the isotopic species were provided by Toth (2000). The spectrum analysis is described in more detail in Supplement 1.

The FTIR analyzer was calibrated for CO$_2$, CH$_4$, CO, N$_2$O, and $^{13}$C in CO$_2$ by measurements of three whole-air reference gases in the laboratory before and after the campaign, following procedures detailed in Griffith et al. (2012). The reference standards were supplied by CSIRO GASLAB, with values for trace gases and $^{13}$C in CO$_2$ traceable to the respective World
Meteorological Organization (WMO) standards for clean air. In addition, a single clean air target tank with amounts of trace gases and $^{13}$CO$_2$ calibrated against the three GASLAB standards was analyzed periodically in the field; these measurements were used to check calibration repeatability and drift, which were <1 nmol mol$^{-1}$ (1 ppb) for N$_2$O and <1 μmol mol$^{-1}$ (1 ppm) for CO$_2$ during the course of the campaign. Constant calibration factors were therefore used for all measurements. For N$_2$O isotopologues, we did not have any reference standards and the calibration was based on the line parameter data and spectrum fit, with relative precision of <0.5 nmol mol$^{-1}$ and an absolute accuracy that was not quantified but normally <2 to 3% (Smith et al., 2011). In summary, for parent 28-N$_2$O, the repeatability was better than 0.5 nmol mol$^{-1}$ and absolute uncertainty was <1 nmol mol$^{-1}$ for the 1-min average measurements; for 29- and 30-N$_2$O isotopologues, 1 σ repeatability was also better than 0.5 nmol mol$^{-1}$ and the accuracy assumed to be better than 3%. For CO$_2$ measurements, the repeatability was better than 0.2 μmol mol$^{-1}$ and absolute uncertainty better than 1 μmol mol$^{-1}$.

The calibration standards spanned mole fractions of 313 to 347 nmol mol$^{-1}$ for N$_2$O with natural isotopic abundances. The 28-N$_2$O mole fractions in chambers ranged up to 3000 nmol mol$^{-1}$, for which no traceable standard was available; however, the FTIR analyzer is known to be highly linear across a wide range of mole fractions (Griffith et al., 2011, 2012), and we have assumed linearity of the calibration across the whole range. Mole fractions of the 29- and 30-N$_2$O isotopologues ranged up to 400 nmol mol$^{-1}$ in the chambers, and we relied on the spectrum fitting procedure for accuracy.

The FTIR measurements of the isotopomers $^{15}$N$^{14}$N$^{16}$O and $^{14}$N$^{15}$N$^{16}$O were identical within measurement accuracy; in the absence of calibration standards for the individual isotopomers at these enrichment levels, our measurements did not discriminate the site preference of $^{15}$N substitution. We therefore combined the two isotopomers and report the combined result as mass 29-N$_2$O ($^{15}$N$^{14}$N$^{16}$O + $^{14}$N$^{15}$N$^{16}$O), as well as 28-N$_2$O ($^{14}$N$^{14}$N$^{16}$O) and 30-N$_2$O ($^{15}$N$^{15}$N$^{16}$O).

The flux of each species was calculated from the change in its concentration $C$ vs. time $t$ during chamber closure by a least squares fit of the concentration–time data from 2 min after closure to 2 min before opening (minutes 8–22 of each 30-min period):

$$ C = C_0 + \frac{FA}{v_{exch}} \left[1 - \exp\left(-\frac{v_{exch}t}{V_{chamber}}\right)\right] $$

where $C_0$ is the initial (background) concentration, $F$ is the flux, $A$ is the chamber area, $V_{chamber}$ is the chamber volume, and $v_{exch}$ is the rate of exchange of chamber air with the atmosphere in an imperfectly sealed chamber. In the limit that $v_{exch}$ or $t$ is small (nonleaky chamber or the initial time after closure), Eq. [3] reduces to a simple linear increase:

$$ C = C_0 + \frac{FA}{V_{chamber}} \cdot t $$

In Eq. [3], $C$ is corrected by approximately 4% for the effect of the FTIR cell volume during measurement; the derivation of Eq. [3], the correction, and examples of fitted growth curves are shown in Supplement 2 (see also Meyer et al., 2001).

The relative amounts of the 28-, 29-, and 30-N$_2$O isotopologues produced depend on the degree to which added $^{15}$N combines with the resident soil $^{14}$N pool to produce N$_2$O. For example, if there is no mixing, all $^{15}$N must be emitted as 30-N$_2$O, all $^{14}$N from the resident pool as 28-N$_2$O, and no 29-N$_2$O is emitted. If the added $^{15}$N mixes completely with the resident $^{14}$N pool available for N$_2$O production, we would expect a statistical distribution of 28-, 29-, and 30-N$_2$O in relative amounts dependent on the atom fraction of $^{15}$N in the mixed pool. The relative distribution of 28-N$_2$O, 29-N$_2$O, and 30-N$_2$O fluxes can thus be used to estimate the fraction of the resident $^{15}$N pool into which the added $^{15}$N is mixed to produce N$_2$O. We denote this fraction as $\alpha$ and calculate its value as

$$ \alpha = \left(\frac{F_{29-N_2O}}{F_{28-N_2O}F_{30-N_2O}}\right)^2 $$

(see Supplement 3 for derivation). Higher values of $\alpha$ (range 0–1) indicate that the added $^{15}$N mixes with a larger proportion of the resident $^{14}$N pool available for N$_2$O production.

Statistical Analysis

Differences in mean CO$_2$ and N$_2$O fluxes before and after N addition were evaluated with a Wilcoxon nonparametric test for treatment and control chambers during both trials using PROC NPARIWAY in SAS (SAS System for Windows, SAS Institute).

Results

Environmental Conditions

Figure 1 depicts air temperature values that ranged from 8 to 26°C during Trial 1 and from 8 to 31°C during Trial 2 and soil temperature values that ranged from 15 to 25°C during Trial 1 and from 8 to 31°C during Trial 2. Similar to air and soil temperatures, daytime net radiation and photosynthetically
active radiation (PAR) were also slightly higher during Trial 2 than during Trial 1. Average net radiation was 420 W m⁻² for Trial 1 and 453 W m⁻² for Trial 2. Average daytime PAR was 944 μE m⁻² s⁻¹ for Trial 1 and 967 μE m⁻² s⁻¹ for Trial 2. Total rainfall (both simulated and natural) was 6.4 cm for Trial 1 and 10.0 cm for Trial 2. Nearly one-half (3.0 cm) of the total rainfall during Trial 1 occurred during a 24-h period on 19 Dec. 2011 (2 d following N addition). A similar event (2.7 cm) occurred during Trial 2 on 8 Jan. 2012 (14 d before N addition). Subsequent Trial 2 rainfall events rarely exceeded 0.3 cm (Fig. 2).

The relationship between WFPS determined from soil cores and moisture sensor data (Fig. 2) collected at the datalogger were fitted ($R^2 = 0.86$) with

$$y = 4.6516 \exp(0.0693x)$$

where $x$ represents the average moisture probe data logged during the 30-min interval when soil samples were collected and $y$ represents predicted WFPS percentage. Figure 2 depicts the measured soil WFPS, which ranged from 30 to >55%, and predicted WFPS, which ranged from 27 to 58% from November through January. The soil WFPS tended to remain above 40% during Trial 1 but tended to drop below 40% during most of Trial 2. Peaks in the soil WFPS occurred during and after rainfall events and dropped precipitously as the many pores in the highly aggregated organic horizon drained rapidly shortly after rainfall ceased.

**Soil Carbon Dioxide Fluxes before and after Nitrogen Addition**

As shown in Fig. 3a and 4a, CO₂ fluxes were almost always positive because plants were clipped weekly inside each chamber and the chambers were dark during closure. We collected respiration data in the absence of photosynthesis because our focus was on belowground activity, and we used these soil respiration data to indicate the presence or absence of microbial priming (Kuzyakov et al., 2000). The average CO₂ flux by chamber during Trial 1 ranged from 0.18 to 0.23 mg CO₂ m⁻² s⁻¹, with an overall chamber average (SD in parentheses) of 0.20 (0.06) mg CO₂ m⁻² s⁻¹. Average CO₂ flux by chamber during Trial 2 ranged from 0.16 to 0.24 mg CO₂ m⁻² s⁻¹, with an overall chamber average of 0.19 (0.05) mg CO₂ m⁻² s⁻¹. Figures 5a and 5b show average CO₂ flux by chamber before and after N addition, with similar patterns during both trials. Those chambers with higher CO₂ emissions before N addition were higher in CO₂ emissions after N addition. Differences in mean flux before vs. after N addition by chamber were not statistically significant for either trial ($P > 0.15$). Cumulative CO₂ fluxes for both trials (before and after N addition) ranged from 400 to 600 g CO₂ m⁻², for an average daily flux ranging from 14 to 22 g CO₂ m⁻² d⁻¹ (Fig. 3a and 4a).

**Soil 28-Nitrous Oxide Fluxes before and after Nitrogen Addition**

Only 28-N₂O fluxes were measured before N addition because 29-N₂O and 30-N₂O isotopologues were below the detection limit in the absence of 15N enrichment. Figures 5c and 5d depict the average 28-N₂O fluxes by chamber before and after N addition, which ranged from 5 to 50 ng N m⁻² s⁻¹. Average 28-N₂O fluxes were slightly higher after N addition for most chambers in both trials; however, the average control chamber 28-N₂O fluxes before and after N addition were similar during Trial 1 but not during Trial 2 (Fig. 5c and 5d). From Fig. 5, fluxes of CO₂ and N₂O appear slightly higher following 15N addition, which may be due to the regular addition of water following N addition and higher soil moisture; however, differences in mean N₂O flux by chamber before and after N addition were not significant for either trial ($P > 0.73$).
Nitrous Oxide Isotopologue Fluxes after Nitrogen-15 Addition and Mixing of Nitrogen-15 and Nitrogen-14 Pools

Figures 6, 7b, and 7c summarize the measured fluxes of individual isotopologues for each trial, and Fig. 8 and 9 show the cumulative fluxes of total $^{15}$N and $^{14}$N as N$_2$O. Table 2 summarizes the cumulative fluxes of all isotopologues (as amounts and as percentages) for each trial and chamber, together with the atom fraction of $^{15}$N in the emitted N$_2$O and the calculated mean value of $\alpha$ (the fraction of resident $^{14}$N with which the $^{15}$N is mixed).

Emissions of $^{15}$N-N$_2$O isotopologues were evident immediately following $^{15}$N addition in both trials. In Trial 1, 29-N$_2$O and 30-N$_2$O emissions were higher than in Trial 2 but subsided below the detection limit within 7 d. In Trial 2, 30-N$_2$O emissions were below the detection limit within 6 d, but 29-N$_2$O emissions remained observable for 10 d. As expected, the largest fraction of N$_2$O was emitted as 28-N$_2$O, where the source of N was derived only from the resident soil $^{14}$N pool.

For the chambers with 0.4 g m$^{-2}$ added $^{15}$N (i.e., all except T1-N1 and T1-N2), the atom fraction of $^{15}$N in the cumulative total N$_2$O ranged from 3.2 to 12.7% (Table 2). The atom fraction of $^{15}$N was higher for those chambers where 0.8 g $^{15}$N m$^{-2}$ was added (11.7–17.4%). In Trial 2, the atom fraction was approximately half that of Trial 1 (5.2 vs. 10.7%). These fractions in N$_2$O are broadly consistent with the fraction of added $^{15}$N relative to the estimated resident inorganic soil $^{14}$N pool (12% at 0.4 g m$^{-2}$ and 24% at 0.8 g m$^{-2}$).

Figures 6 and 7a show the time course of the calculated mixing fraction ($\alpha$) throughout each trial, and Table 2 summarizes the mean values of $\alpha$ from the cumulative data. The time courses show how $\alpha$ began near 0.1 shortly after $^{15}$N addition and increased to near 0.5 after rainfall or supplementary water additions. The initial pulses of N$_2$O emissions, $\alpha$ became increasingly “noisy” due to the uncertainties in the low fluxes but stayed near or below 0.5 until it became undefined as the fluxes approach zero.

Thus, the added $^{15}$N initially mixed with only $\sim$10% of the resident $^{14}$N soil pool, increasing to $\sim$50% after rainfall or watering. The average mixing value ranged from 17% to 52% during each measurement period. This led to higher relative amounts of 30-N$_2$O than would be expected if complete mixing between the added and resident pools of N were assumed.

Nitrogen-15 Recovery

Tables 3 and 4 and Fig. 10 summarize the recoveries of $^{15}$N in the various pools at the end of the trials. The total amount of $^{15}$N recovered as N$_2$O for each chamber was <11 mg N m$^{-2}$ for Trial 1 and <4 mg N m$^{-2}$ for Trial 2, compared with 110 and 80 mg N m$^{-2}$ for $^{14}$N (Table 3). Small rises in $^{14}$N-N$_2$O fluxes following N addition were similar for both amended and control chambers, presumably influenced by the added water (Fig. 6 and 7). The form of $^{15}$N added did not produce unique patterns in isotopologue fluxes or pools of $^{15}$N recovered for either trial (Fig. 8, 9, and 10).

The average percentage of $^{15}$N recovered as N$_2$O across both trials was 1% (range 0.7–1.9%), and labeled N was no longer evident as N$_2$O within 10 d following label addition. The
Fig. 5. (a,b) Average CO$_2$ and (c,d) $^{14}$N$^{15}$N$^{16}$O (28-N$_2$O) fluxes by chamber before and after N addition by trial. Chamber abbreviations on the x axis refer to Trial 1 (T1) or Trial 2 (T2) and treatment (urea [U] or NO$_3$ [N]).

Fig. 6. Trial 1 N$_2$O isotopologue fluxes collected every 30 min for each chamber measured from 2 to 28 Dec. 2011: (a) fraction $\alpha$ of added $^{15}$N that mixed and reacted with the $^{14}$N pool (see Eq. [5]); (b) fluxes of $^{15}$N$^{15}$N$^{16}$O (30-N$_2$O); and (c) fluxes of $^{16}$N$^{15}$N$^{16}$O + $^{15}$N$^{14}$N$^{16}$O (29-N$_2$O); y-axis labels are shown in the center of each graph for clarity.
aboveground shoot biomass and the proportion of $^{15}$N recovered in shoots at the end of each trial was greater for Trial 1, while the belowground root biomass and the proportion of $^{15}$N recovered in roots was greater for Trial 2 (Table 3; Fig. 10). Root/shoot ratio values for Trial 2 were more than double the root/shoot ratios for Trial 1 (Table 4). The percentages of $^{15}$N in roots and shoots ranged between 0.9 and 2.15% and between 3.14 and 4.21%, for Trials 1 and 2, respectively. The variability in $^{15}$N recovered in the microbial biomass was high (6.8–30.3 mg m$^{-2}$) for both trials. The average (SD in parentheses) microbial C/N ratio was 10.18 (1.05) for Trial 1 and 20.18 (3.49) for Trial 2 (Table 4). Recovery of $^{15}$N in plant material (both roots and shoots) averaged 21% (4%) for Trial 1 and 18% (7%) for Trial 2 (Table 3). Plant $^{15}$N values (both roots and shoots) were similar among chambers for both trials, including the control chambers. Figure 10 illustrates how the relative percentages of total $^{15}$N recovered by pool varied for Trials 1 and 2. The trend for greater plant $^{15}$N uptake for chambers amended with KNO$_3$ seen in Trial 1 was not evident for Trial 2.
Discussion

Fluxes of N₂O are strongly controlled by environmental conditions (Attard et al., 2011), and we found greater fluxes for all isotopologues (28-N₂O, 29-N₂O, and 30-N₂O) during and following rainfall events. Further, peaks in WFPS (Fig. 2) were followed by peaks in all N₂O isotopologue fluxes during both trials (Fig. 3 and 4). A large body of work has demonstrated the importance of WFPS in predicting 28-N₂O emissions (Bateman and Baggs, 2005; Hénault et al., 2005; Klumpp et al., 2011); however, rarely are the relationships between WFPS and 29-N₂O and 30-N₂O fluxes reported in the field. The synchronous nature of emission peaks among the N₂O isotopologues during both trials (Fig. 3, 4, 6, and 7) indicated that rainfall consistently stimulated emissions of 28-N₂O, 29-N₂O, and 30-N₂O isotopologues, despite spatial variability in the magnitude of N₂O fluxes depicted in Fig. 8 and 9.

The greatest N₂O emissions were observed when WFPS values rose above 50%, and these high WFPS conditions occurred only during Trial 1 between 19 and 22 December (Fig. 3). At WFPS values between 50 and 60%, the presence of aerobic and anaerobic microsites would favor both nitrification and denitrification processes, as reported by Well et al. (2006) and Ostrom et al. (2010). The contribution by each process in the field, however, varies with soil characteristics (Pérez et al., 2006; Toyoda et al., 2011), soil management (Toyoda et al., 2011), substrate availability (Attard et al., 2011), and the WFPS value (Bateman and Baggs, 2005; Klumpp et al., 2011). Further, while

![Fig. 9. Trial 2 cumulative fluxes for (a) 15N-N₂O and (b) 14N-N₂O for each chamber. Two chambers are shown for each treatment and one water-only control; y-axis labels are shown in the center of each graph for clarity.](image)

<table>
<thead>
<tr>
<th>Chamber†</th>
<th>Added ³⁵N</th>
<th>Cumulative N₂O Flux‡</th>
<th>Atom% ³⁵N in N₂O</th>
<th>σ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g N m⁻²</td>
<td>Content</td>
<td>Concentration</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mg N₂O m⁻²</td>
<td>30-N₂O</td>
<td>29-N₂O</td>
<td>28-N₂O</td>
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<td>13.3</td>
<td>69.8</td>
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<tr>
<td>T1-C</td>
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<td></td>
<td></td>
<td>21.6</td>
</tr>
<tr>
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<td>2.5</td>
<td>12.2</td>
<td>57.8</td>
</tr>
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<td>0.8</td>
<td>4.6</td>
<td>33.6</td>
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<tr>
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<td>0.6</td>
<td>7.1</td>
<td>76.9</td>
</tr>
<tr>
<td>T2-U1</td>
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<td>0.8</td>
<td>6.1</td>
<td>66.9</td>
</tr>
<tr>
<td>T2-U2</td>
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<td>2.6</td>
<td>44.2</td>
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<tr>
<td>T2-C</td>
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<td></td>
<td>48.2</td>
</tr>
<tr>
<td>Avg.</td>
<td></td>
<td>0.6</td>
<td>5.1</td>
<td>55.4</td>
</tr>
</tbody>
</table>

† T1, Trial 1; T2, Trial 2; N, NO₃ treatment; U, urea treatment; C, control.
‡ 30-N₂O, ³¹N³¹N₁⁶O; 29-N₂O, ³¹N³²N₁⁶O + ³²N³¹N₁⁶O; 28-N₂O, ³²N³²N₁⁶O.

Table 2. Cumulative fluxes by chamber after N addition for each isotopologue and trial, by amount and by fraction. Atom% ³⁵N in N₂O refers to the atom fraction of ³⁵N in all emitted N₂O; σ refers to the fraction of the resident ¹⁴N pool into which the added ³⁵N mixes to form N₂O.
WFPS measured at 6 cm did not rise above 60%, it is possible that greater WFPS levels occurred below 6 cm. During Trial 2, WFPS values rarely exceeded 40%, and the magnitudes of the N₂O fluxes were considerably lower (Fig. 4). Substantial drying occurred before Trial 2, when the soil temperature exceeded 25°C for several days between 20 December and 7 January. Repeated rainfall simulation events (Fig. 2) did not increase WFPS to the levels found in Trial 1. We suspect that higher WFPS during Trial 1 would have favored N₂O produced by denitrification more than during Trial 2, when WFPS rarely rose above 40% (Fig. 2, 3, and 4). These results point to the importance of WFPS measurement when interpreting differences in the magnitude of N₂O emissions following rainfall events.

Before ¹⁵N additions, background fluxes of CO₂ and ²⁸-N₂O were measured, although fluxes of 29-N₂O and ³⁰-N₂O were below the detection limit (Fig. 6, 7, 8, and 9). Immediately following KNO₃ or CO(NH₂)₂ addition, we found that the transformation of soluble N to N₂O occurred rapidly (Klumpp et al., 2011); however, we did not find consistent differences in N₂O fluxes associated with the N source [KNO₃ (Klumpp et al., 2011); however, we did not find consistent differences in N₂O fluxes associated with the N source [KNO₃ or CO(NH₂)₂] at these applied levels of N. Instead, we found unique isotopologue profiles in two trials only 1 mo apart, with lower percentages of cumulative ¹⁵N-N₂O emissions in January (5%) than December (10%, Table 2). In addition, a greater fraction of applied N remained in the soil pool, despite frequent simulated rainfall events, and more ¹⁵N was allocated to roots (Fig. 10). While the WFPS percentage tends to be the dominant factor influencing N₂O emissions (Well et al., 2006), other plant–microbial interactions associated with seasonal changes could have contributed to lower N₂O emissions, but this remains to be tested. All in all, the average fraction of ¹⁵N applied that was emitted as ¹⁵N-N₂O (~1%) was within IPCC estimates (Intergovernmental Panel on Climate Change, 2006) for this very limited time period and at low agronomic levels of N addition.

Peaks in N₂O emissions following the application of N reportedly persist for several weeks (Breitenbeck and Bremmer, 1986; Chantigny et al., 1998; Bouwman et al., 2002; Phillips, 2007). In the absence of a method for tracking added N, all N₂O peaks following fertilizer N application are often attributed to the added N. In this study, we found that emissions of ¹⁵N-N₂O persisted for days instead of weeks at low levels of N addition, which is similar to grassland work reported by Klumpp et al. (2011). Most ¹⁵N-N₂O and ¹³N-N₂O emissions occurred during

### Table 3. Recovery of ¹⁵N in soil, root, shoot, and microbial biomass pools for each trial and chamber. Recovery of ¹⁵N-N₂O was calculated based on cumulative fluxes collected continuously for 2 wk. Total recovery includes estimated denitrified-N losses to the atmosphere, based on a N₂/O/N₂ ratio of 0.6 (Rudaz et al., 1999; Mathieu et al., 2006; Bergstermann et al., 2011).

<table>
<thead>
<tr>
<th>Trial</th>
<th>Chamber†</th>
<th>Soil ¹⁵N</th>
<th>Root ¹⁵N</th>
<th>Shoot ¹⁵N</th>
<th>Microbial ¹⁵N</th>
<th>¹⁵N-N₂O Total</th>
<th>Estimated ¹⁵N-N₂O</th>
<th>Recovered ¹⁵N-N₂O</th>
<th>Recovered total ¹⁵N</th>
<th>K₂SO₄-extractable ¹⁵N‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T1-N1</td>
<td>205.4</td>
<td>47.1</td>
<td>175.8</td>
<td>6.8</td>
<td>10.7</td>
<td>445.8</td>
<td>17.9</td>
<td>2.5</td>
<td>54.0</td>
</tr>
<tr>
<td>1</td>
<td>T1-N2</td>
<td>167.0</td>
<td>25.0</td>
<td>121.0</td>
<td>9.2</td>
<td>10.4</td>
<td>332.2</td>
<td>17.3</td>
<td>2.4</td>
<td>40.7</td>
</tr>
<tr>
<td>1</td>
<td>T1-U1</td>
<td>202.5</td>
<td>13.1</td>
<td>83.6</td>
<td>14.0</td>
<td>10.0</td>
<td>323.2</td>
<td>16.6</td>
<td>4.0</td>
<td>67.6</td>
</tr>
<tr>
<td>1</td>
<td>T1-U2</td>
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<td>39.9</td>
<td>68.8</td>
<td>30.3</td>
<td>4.2</td>
<td>351.7</td>
<td>6.9</td>
<td>1.7</td>
<td>71.3</td>
</tr>
<tr>
<td>1</td>
<td>T1-C</td>
<td>0.0</td>
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<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

†T1, Trial 1; T2, Trial 2; N, NO₃ treatment; U, urea treatment; C, control.
‡Extractable ¹⁵N represents the inorganic and organic ¹⁵N recovered in unfumigated, 0- to 10-cm-depth samples and not additional ¹⁵N recovery (already included in soil and microbial ¹⁵N).

### Table 4. Total aboveground and belowground plant biomass and microbial biomass (0–10 cm) harvested 2 wk following ¹⁵N addition for each trial and chamber. Most root mass (>90%) was found in the upper 10 cm.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Chamber†</th>
<th>Root mass, 0–20 cm</th>
<th>Shoot mass</th>
<th>Root/shoot ratio</th>
<th>Root N %</th>
<th>Shoot %N</th>
<th>Bulk soil N 0–20 cm</th>
<th>Microbial C</th>
<th>Microbial N</th>
<th>Microbial C/N ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T1-N1</td>
<td>119.3</td>
<td>81.4</td>
<td>1.5</td>
<td>1.37</td>
<td>3.14</td>
<td>444.6</td>
<td>977.9</td>
<td>114.2</td>
<td>8.6</td>
</tr>
<tr>
<td>1</td>
<td>T1-N2</td>
<td>72.4</td>
<td>60.3</td>
<td>1.2</td>
<td>1.22</td>
<td>3.32</td>
<td>556.4</td>
<td>864.5</td>
<td>75.6</td>
<td>11.4</td>
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<td>T1-U1</td>
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<td>1.16</td>
<td>3.80</td>
<td>462.6</td>
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<td>126.8</td>
<td>10.3</td>
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<td>0.89</td>
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<td>565.2</td>
<td>1459.2</td>
<td>135.8</td>
<td>10.7</td>
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<td>1</td>
<td>T1-C</td>
<td>50.0</td>
<td>49.4</td>
<td>1.0</td>
<td>1.12</td>
<td>3.32</td>
<td>547.8</td>
<td>1382.3</td>
<td>140.2</td>
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<td>1.29</td>
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<td>493.7</td>
<td>1857.4</td>
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<td>1.55</td>
<td>3.63</td>
<td>554.7</td>
<td>1538.7</td>
<td>67.9</td>
<td>22.6</td>
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<td>1911.0</td>
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<td>1883.2</td>
<td>76.9</td>
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<td>382.5</td>
<td>1842.7</td>
<td>108.6</td>
<td>17.0</td>
</tr>
</tbody>
</table>

†T1, Trial 1; T2, Trial 2; N, NO₃ treatment; U, urea treatment; C, control.
Trial 1 following a 24-h rainfall event (2 d after $^{15}$N addition) and subsequently leveled off (Fig. 3, 6, and 8) despite small rainfall events later in December (Fig. 2). The effect of rainfall events on all $^{15}$N fluxes also diminished with time for Trial 2 (Fig. 4, 7, and 9), despite drier conditions and repeated rainfall (natural and simulated) events (Fig. 2). The high proportion of $^{15}$N recovered as $^{15}$N-$\text{N}_2\text{O}$ (relative to resident inorganic $N$) reported in Table 2 could be explained by the high availability of applied $^{15}$N to microbes under moist conditions and incomplete mixing with the resident $N$ pool. Both $^{15}$N-$\text{N}_2\text{O}$ and $^{14}$N-$\text{N}_2\text{O}$ emissions were substantive following rainfall when WFPS was $>$35%, suggesting that resident $N$ at the site was less available than dissolved $^{15}$N for $\text{N}_2\text{O}$ production. Although additional $^{15}$N-$\text{N}_2\text{O}$ emissions could have occurred beyond our 14-d observation period, large peaks of $^{15}$N-$\text{N}_2\text{O}$ would not be expected. In a 160-d study, Klumpp et al. (2011) found that $^{15}$N-$\text{N}_2\text{O}$ peaks diminished dramatically within 10 d, and $^{15}$N-$\text{N}_2\text{O}$ was no longer evident after 30 d.

Soil respiration data are used to indicate priming or lack of priming effects resulting from substrate additions (Jenkinson et al., 1985; Hart et al., 1986). Statistical analyses were limited by the small number of chambers in this study (Blagodatsky and Smith, 2012; Bastida et al., 2013), but our results indicate that actual priming (enhanced soil respiration) did not occur. The apparent increase in $\text{CO}_2$ and $\text{N}_2\text{O}$ mean fluxes after $N$ addition (Fig. 5) may have been due to greater WFPS (Fig. 2). Soil $\text{CO}_2$ fluxes did not spike following trace $N$ addition, and plant $N$ was similar among chambers (including the water-only control) for both trials. These results suggest that $N$ addition did not actually prime biogenic activity (Jenkinson et al., 1985). Instead, some resident soil $^{14}N$ exchanged with added $^{15}N$ (pool substitution), which is an apparent priming effect (Kuzyakov et al., 2000). Pool substitution can only occur if added $^{15}N$ and resident $^{14}N$ occupy the same pool (Hart et al., 1986), so the $^{15}N$ added in these experiments needed to mix with soil $^{15}N$ to facilitate this substitution (Bergstermann et al., 2011). One way of evaluating the degree to which $^{15}N$ and resident $^{14}N$ physi cally mixed and reacted with each other is given by $\alpha$ (Eq. [5]). The fraction of resident $^{14}N$ into which the added $^{15}N$ was transported and converted to $\text{N}_2\text{O}$ ($\alpha$) was initially low ($\approx$0.1) but increased to approximately 0.5 following rainfall events, which suggests a more homogeneous distribution of added and resident $N$ pools during rain. We use $\alpha$ to broadly indicate the physical opportunity for interaction among $N$ pools because this is an important component to $N$-addition experiments (Well and Butterbach-Bahl, 2013). Alpha increased for all chambers following substantive rainfall events (Fig. 6 and 7). If $\alpha$ values were very low for some chambers and very high for others, physical mixing constraints alone would influence the spatial variation in $\text{N}_2\text{O}$ isotopologue flux profiles (Fig. 8 and 9).

Similar to the results of Morse and Bernhardt (2013), total recovery of $^{15}N$ was highly variable, ranging from 41.1 to 81.6%. Sources of error include the estimated amount of $^{15}N$ that could have been lost as gaseous $N$ (e.g., $\text{NO}$, $\text{NH}_3$, or $\text{N}_2$), leaching below the 20-cm depth, and nonhomogeneous distribution of added $^{15}N$. We estimated $\text{N}_2$ emissions based on reported $\text{N}_2/\text{N}_2\text{O}$ ratios (Table 3), but this ratio changes dramatically with time and conditions (Firestone and Tiedje, 1979; Pérez et al., 2001; Bergstermann et al., 2011). Low $^{15}N$ recoveries might also be explained by other gaseous-$N$ losses, such as $\text{NO}_x$ and $\text{NH}_3$, not measured in this study. Acid sulfate soil emissions of $\text{NO}_x$ as high as 0.025 g m$^{-2}$ d$^{-1}$ have been reported (Macdonald et al., 2011), as well as $\text{NH}_3$ losses totaling 0.5 to 16% of total applied $N$ (Khanif, 1992). We suspect that the greatest source of variation in the recovery of $^{15}N$ was associated with nonhomogeneous distribution of $^{15}N$ throughout the soil profile (Bergstermann et al., 2011). The soil core collected at the end of each trial was a small fraction of the chamber footprint and may not have represented the entire chamber area.

The percentages of $^{15}N$ recovered in the soil, plant, and microbial biomass pools were different between trials, with greater $^{15}N$ recovered in shoot biomass during Trial 1 and greater $^{15}N$ recovered in root biomass during Trial 2. This may be attributed to differences in plant allocation before and after the summer solstice because solar radiation and temperature conditions were similar during both trials. Alternatively, less mixing ($\alpha$) occurred during Trial 2 (Table 2). If $^{15}N$ was less...
completely mixed in the soil, less $^{15}$N may have been available for shoot growth during Trial 2. Overall, 18 to 21% of the total $^{15}$N applied to the chamber plots was recovered in plant material, which is close to the 25% reported by de Vries et al. (2011). Factors that varied more strongly between trials included lower percentages of $^{15}$N recovered in N$_2$O for Trial 2 and higher microbial C/N ratios for Trial 2 could suggest that microbes were N limited, but this was not likely because plant and soil N values were high (Table 4). One possible factor influencing N cycling that we did not measure was fungal activity. Fungi play an important role in grassland denitrification (Laughlin et al., 2008), and N retention is reportedly higher when the fungal biomass is high (de Vries et al., 2011). Fungi or some other factor could have affected N immobilization after the summer solstice if the seasonality of soil biogenic activities varies among functional groups (Smith et al., 2010), but this would require additional study.

Tracking how agronomic additions of fertilizer N influence N$_2$O isotopologues with this technique will help bridge the knowledge gap between application rate and percentage of N emitted as NO$_3$ in addition to providing guidance for sustainable management practices. Unlike some laboratory-based techniques, this field-based FTIR system cannot resolve natural isotopic abundances of N or O but can resolve 28-N$_2$O, 29-N$_2$O, and 30-N$_2$O at high temporal frequency when $^{15}$N-labeled substrate is applied. Linking the FTIR with automated chambers provides the temporal data needed to capture weather-driven emission peaks in the field. This is important because N$_2$O production is highly episodic and occurs at the soil microsite scale. Detection of N$_2$O isotopologues can point to mobilization of the resident soil N following fertilizer addition to produce N$_2$O and the degree to which N is mixed in the soil. Finally, linking this method with controlled incubation studies using IRMS may be a fruitful way forward to link temporal variability in N$_2$O emissions with microbial processes following agricultural N addition.

Conclusions

We have demonstrated continuous field measurement, using a portable FTIR spectrometer and automated flux chambers, of the emissions of $^{15}$N- and $^{15}$N-N$_2$O isotopologues at the surface of an intact field plot following small (<1 g m$^{-2}$) additions of $^{15}$N as NO$_3$ and urea to the soil. The FTIR analyzer ran for >2 mo continuously in the field and provided repeatability of better than 0.5 nmol mol$^{-1}$ for amounts and flux detection limits of better than 0.1 ng N m$^{-2}$ s$^{-1}$ for all isotopologues. This capability is not available from mass spectrometry based methods, and to our knowledge has not been published for laser-based analyzers.

The synchrony of all N$_2$O isotopologue fluxes with soil moisture was clearly evident during both trials. Quantification of 28-N$_2$O, 29-N$_2$O, and 30-N$_2$O fluxes showed that (i) an average of 1% (range 0.7–1.9%) of the added N was emitted as N$_2$O, which is consistent with the guidelines of the Intergovernmental Panel on Climate Change, (ii) 3 to 13% of the emitted N$_2$O contained the $^{15}$N isotope, which was consistent with the percentage of N added relative to resident inorganic N (~12% at the 0.4 g m$^{-2}$ dose), and (iii) the added $^{15}$N mixed with only 10 to 50% of the resident $^{15}$N pool capable of N$_2$O production, as calculated using the distribution of 28-, 29-, and 30-$^{15}$N$_2$O fluxes.

Future work is needed to quantify how fertilizers affect N$_2$O fluxes under agronomic rates of N application for longer time periods. In addition, exploring how mixing of applied N in the field might influence N available for crop growth and N$_2$O emissions could be a fertile area of research. Additional investigations might include application of dual-labeled CO(NH$_2$)$_2$ ($^{13}$C and $^{15}$N) to elucidate how urea CO(NH$_2$)$_2$ application affects both C and N pools in the field because $^{13}$C-$^14$CO, $^{13}$C-$^15$CO, $^{14}$N-$^15$O, and $^{15}$N-$^14$N can all be resolved with the FTIR system. This technique can provide measurements important for answering agronomic questions and lead to greater understanding of how weather, application, and season of N addition influence N$_2$O emissions in the field at high temporal resolution.

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