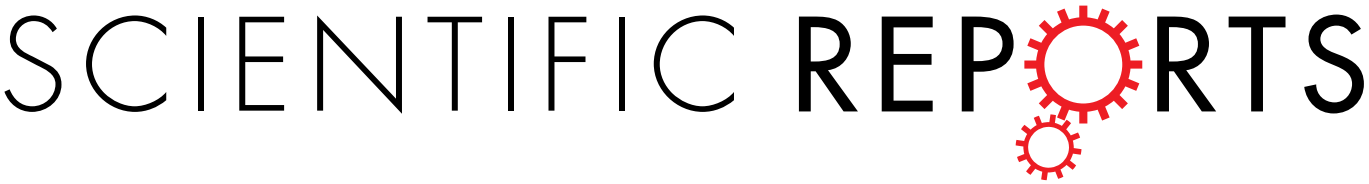

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# Disentangling gross $N_2O$ production and consumption in soil

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**The difficulty of measuring gross $N_2O$ production and consumption in soil impedes our ability to predict $N_2O$ dynamics across the soil-atmosphere interface. Our study aimed to disentangle these processes by comparing measurements from gas-flow soil core (GFSC) and $^{15}N_2O$ pool dilution ($^{15}N_2O$PD) methods. GFSC directly measures soil $N_2O$ and $N_2$ fluxes, with their sum as the gross $N_2O$ production, whereas $^{15}N_2O$PD involves addition of $^{15}N_2O$ into a chamber headspace and measuring its isotopic dilution over time. Measurements were conducted on intact soil cores from grassland, cropland, beech and pine forests. Across sites, gross $N_2O$ production and consumption measured by $^{15}N_2O$PD were only 10% and 6%, respectively, of those measured by GFSC. However, $^{15}N_2O$PD remains the only method that can be used under field conditions to measure atmospheric $N_2O$ uptake in soil. We propose to use different terminologies for the gross $N_2O$ fluxes that these two methods quantified. For $^{15}N_2O$PD, we suggest using 'gross $N_2O$ emission and uptake', which encompass gas exchange within the $^{15}N_2O$-labelled, soil air-filled pores. For GFSC, 'gross $N_2O$ production and consumption' can be used, which includes both $N_2O$ emitted into the soil air-filled pores and $N_2O$ directly consumed, forming $N_2$, in soil anaerobic microsites.**

$N_2O$ is one of the most important long-lived greenhouse gases and is expected to be the single most important ozone-depleting substance throughout the 21st century[1]. Soils account, globally, for about 60% of the total $N_2O$ flux to the atmosphere, with 6.6 Tg N $yr^{-1}$ from natural ecosystems and 4.1 Tg N $yr^{-1}$ from agricultural systems[2]. Although it is generally known that microbial nitrification and denitrification in soils are the major sources of atmospheric $N_2O$, it remains a struggle to disentangle and quantify gross rates of microbial $N_2O$ production and consumption in soil which, in turn, determine the net $N_2O$ flux across the soil-atmosphere interface.

Under anaerobic conditions, incomplete denitrification produces $N_2O$ whereas the terminal step of denitrification (i.e. the reduction of $N_2O$ to $N_2$) consumes $N_2O$. Hence, microbial $N_2O$ production and consumption can occur simultaneously in soil via the activities of different microorganisms or even by a single denitrifying cell[3]. In addition, within the soil profile and in the soil air-filled pores, $N_2O$ can be further reduced to $N_2$ during its transport to the soil surface[4–6]. Soil physical (e.g. water or oxygen content, temperature, porosity) and biochemical factors (e.g. pH, concentrations of electron donors and acceptors) influence the balance between soil $N_2O$ production and consumption[7], and consequently the net $N_2O$ flux to the atmosphere. Soil net $N_2O$ uptake has been compiled in a review[8], which specifically refers to the net flux of $N_2O$ from the atmosphere to the soil and can be detected only if soil $N_2O$ consumption exceeds production. Soil $N_2O$ consumption, however, is often ignored because it is prone to be masked by the much larger $N_2O$ production[4] and is difficult to measure directly (e.g. as soil $N_2$ flux) against a very high (78%) atmospheric background[9].

The static chamber method, commonly used to measure net $N_2O$ flux on the soil surface, cannot quantify the simultaneously occurring gross $N_2O$ production and consumption within the soil. One possibility to measure gross $N_2O$ production and consumption in soil is the $^{15}N_2O$ pool dilution ($^{15}N_2O$PD) technique, which entails adding $^{15}N_2O$ to the chamber headspace and subsequently measuring the changes in $^{14}N_2O$ and $^{15}N_2O$ over time[10]. So far, this $^{15}N_2O$PD technique has been used in managed grassland and cropland soils and in salt marsh landscape, all located in northern California, by the same authors who first evaluated this method under field conditions[10–12].

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| Site characteristics | Grassland | Cropland | Beech forest | Pine forest |
|---|---|---|---|---|
| Location | 47.57°N, 11.03°E | 48.19°N, 11.96°E | 51.76°N, 9.58°E | 43.72°N, 10.28°E |
| Mean annual temperature (°C) | 6.7 | 8.5 | 7.3 | 14.1 |
| Mean annual precipitation (mm) | 1373 | 1029 | 1100 | 918 |
| Elevation (m above sea level) | 870 | 510 | 510 | 10 |
| Vegetation/Crop | *Poaceae*; *Taraxacum* | *Zea mays* | *Fagus sylvatica* | *Pinus pinaster* |
| Soil type | Haplic Cambisol | Calcaric Cambisol | Dystric Cambisol | Calcareous Regosol |
| Soil texture (% sand/silt/clay) | 10/68/23 | 30/52/18 | 12 / 54/34 | 93/3/4 |
| Soil bulk density (g $cm^{-3}$) | 0.59 | 1.17 | 0.64 | 1.30 |
| Soil pH | 7.1 | 6.7 | 3.8 | 5.7 |
| Soil total organic carbon (g C $kg^{-1}$) | 135 | 20 | 127 | 10 |
| Soil total nitrogen (g N $kg^{-1}$) | 8.0 | 1.7 | 6.6 | 0.7 |
| Soil C:N ratio | 16.9 | 11.8 | 18.9 | 13.5 |

**Table 1. Site characteristics.** Soil characteristics in the grassland, cropland and pine forest sites were measured in the top 10 cm of mineral soil[19,21]; in the beech forest site, these were measured in the top 5 cm of mineral soil.

In 2013, when the first $^{15}N_2OPD$ measurements were reported[10], a debate emerged as to what extent this technique is able to quantify gross $N_2O$ production and consumption in soil. Well & Butterbach-Bahl[13] questioned the key assumptions of the $^{15}N_2OPD$ technique: the exchange and mixing of soil-derived $N_2O$ and $^{15}N_2O$ label between aerobic and anaerobic soil microsites. They argued that gross $N_2O$ production and consumption in soil would be underestimated if produced $N_2O$ was immediately reduced to $N_2$ without first mixing with the $^{15}N_2O$-labelled air in interconnected soil pore spaces. This may occur within denitrifier cells and between different microorganisms[3] in anaerobic microsites, which here we infer to include not only microsites saturated with water but also isolated pores filled with or enclosed by water and water-entrapped $N_2O$[14]. Yang *et al.*[15] replied that such constraints could only occur when the soil has a high proportion of anaerobic microsites, and argued that the $^{15}N_2O$ label and soil-derived $N_2O$ are likely distributed homogeneously in the chamber headspace from which the calculation of gross $N_2O$ fluxes is derived. In summary, the efficacy of the $^{15}N_2OPD$ technique to estimate gross $N_2O$ production and consumption is still not settled, and so far this technique has only been compared with results from acetylene inhibition and $^{15}N$ tracing methods. These latter methods, however, have their own limitations for determining gross $N_2O$ production and consumption in soil since they either modify the entire denitrification process as well as its single steps (acetylene inhibition method) or require the addition of $^{15}N$-labelled substrate ($^{15}N$ tracing method) with the need to label the soil homogeneously including its anaerobic microsites[9,16].

To date, the enigmatic lack of measurements of gross $N_2O$ production and consumption in soil impedes our ability to predict $N_2O$ dynamics across the soil-atmosphere interface. Our study aimed to disentangle gross $N_2O$ production and gross $N_2O$ consumption in soil by comparing measurements from $^{15}N_2OPD$ technique and gas-flow soil core (GFSC) method. The latter is an established method that directly measures gross $N_2O$ production and consumption in soil by simultaneously quantifying $N_2O$ and $N_2$ fluxes[17] without the use of an inhibitor or $^{15}N$ labelling of substrate[9,16]. We hypothesized that if the assumption of the $^{15}N_2OPD$ method (i.e. exchange and mixing of soil-derived $N_2O$ and $^{15}N_2O$ label between aerobic and anaerobic soil microsites) is attained, then the $^{15}N_2OPD$ and GFSC methods should yield comparable estimates of gross $N_2O$ production and consumption in soil. We tested this hypothesis using different soils from four ecosystems: grassland, cropland, beech and pine forests (Table 1), covering a range of soil biochemical characteristics as well as soil aeration status (e.g. water content and soil texture) and N availability.

## Results

From the $^{15}N_2OPD$ measurements, gross $N_2O$ production and consumption rates and net $N_2O$ flux (Fig. 1a–c) were higher ($p = 0.01$–$0.03$) in the silty loam Cambisol soil in manured grassland than in the sandy Regosol soil in unmanaged pine forests, and neither differed from the sandy loam Cambisol soil in cropland or the silty loam Cambisol soil in unmanaged beech forest. For the grassland, cropland and beech forest, net $N_2O$ emissions accounted for 66–79% of gross $N_2O$ production (Fig. 1d). For the pine forest, net $N_2O$ uptake (Fig. 1c) was paralleled by larger gross $N_2O$ consumption (Fig. 1b) than gross $N_2O$ production (Fig. 1a); these fluxes were very small but still above our detection limit.

From the GFSC measurements, gross $N_2O$ production (Fig. 1a) was higher ($p = 0.02$) in the beech forest than in the cropland and pine forest and intermediate in the grassland. Gross $N_2O$ consumption ($p = 0.37$; Fig. 1b) and net $N_2O$ fluxes ($p = 0.06$; Fig. 1c) did not differ among sites. Net $N_2O$ fluxes accounted, on average, for only 24% of gross $N_2O$ production (Fig. 1d), and hence most (76%) of the produced $N_2O$ was further reduced to $N_2$.

Although significant differences in gross $N_2O$ production and consumption between the $^{15}N_2OPD$ technique and GFSC method were only found in the grassland site ($p = 0.02$ for both; Fig. 1a,b), the fluxes measured by the GFSC method were up to two orders of magnitude larger than those measured by the $^{15}N_2OPD$ technique (Fig. 1a,b). The large spatial variation within each site (indicated by the large standard errors) resulted in non-statistically detectable differences between these two methods. However, for gross $N_2O$ production, rates measured by the $^{15}N_2OPD$ technique were on average 10% of those measured by the GFSC method (Fig. 1a). For gross

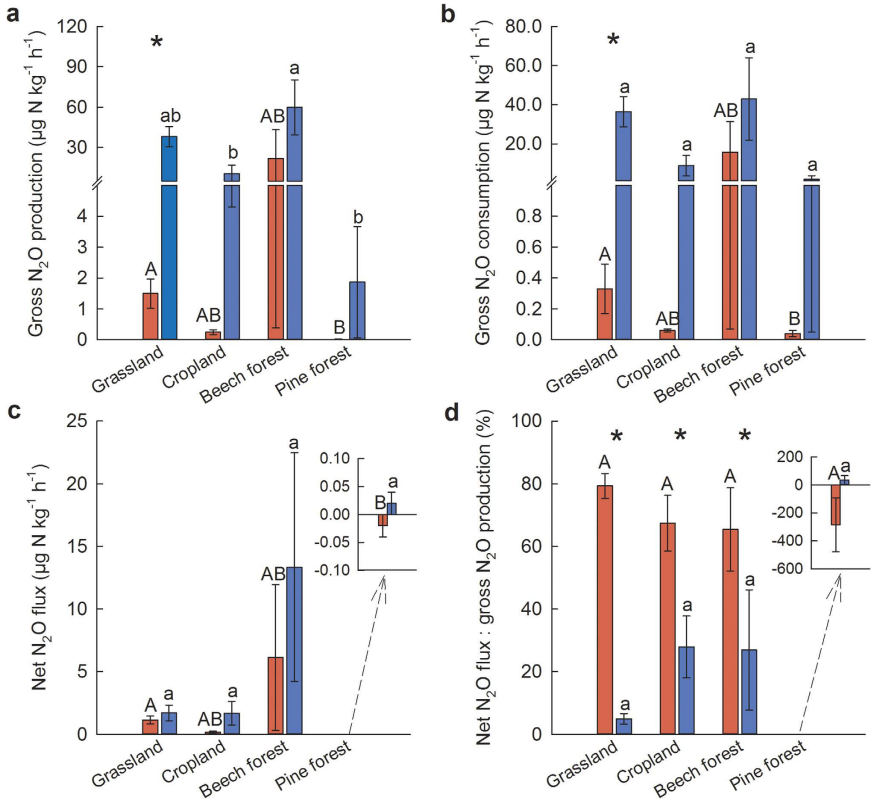


**Figure 1. Soil gross and net $N_2O$ fluxes.** Gross $N_2O$ production (**a**), gross $N_2O$ consumption (**b**), net $N_2O$ flux (**c**), and the ratio of net $N_2O$ flux to gross $N_2O$ production (**d**), measured by $^{15}N_2O$ pool dilution ($^{15}N_2OPD$; red bars) and gas-flow soil core (GFSC; blue bars). For each method, means (± s.e., n = 4 replicate sampling points) with different capital (for $^{15}N_2OPD$) and small letters (for GFSC) indicate significant differences among sites (one-way ANOVA with Fisher's LSD test at $p \leq 0.05$ or Kruskal-Wallis ANOVA with multiple comparisons of mean ranks at $p \leq 0.05$). For each site, asterisks above the bars indicate significant differences between the two methods (paired t test at $p \leq 0.05$).

$N_2O$ consumption, rates measured by the $^{15}N_2OPD$ technique were on average 6% of those measured by the GFSC method (Fig. 1b). Net $N_2O$ fluxes from the soil cores used for the $^{15}N_2OPD$ measurement were on average 94% of those measured by the GFSC method, which did not differ in any of the sites ($p = 0.11$–$0.61$; Fig. 1c). In three sites, except in the pine forest that had very low fluxes, the ratios of net $N_2O$ flux to gross $N_2O$ production measured by the $^{15}N_2OPD$ technique were higher ($p < 0.01$–$0.05$) than those measured by the GFSC method (Fig. 1d).

Soil water-filled pore space (WFPS), microbial C and N, and denitrification enzyme activity (DEA) were generally higher ($p \leq 0.02$) in the grassland than in the pine forest (Table 2). Soil $NH_4^+$ concentrations were higher ($p < 0.01$) in the grassland and beech forest compared to the cropland, whereas soil $NO_3^-$ concentrations were higher ($p = 0.02$) in the cropland than in the grassland and pine forest (Table 2). Gross $N_2O$ production and consumption, measured by either the $^{15}N_2OPD$ technique or the GFSC method, showed positive correlations with WFPS, $NH_4^+$, microbial C and N, and DEA (R = 0.56–0.93, $p < 0.05$; Supplementary Table S1). Net $N_2O$ fluxes from the soil cores used for the $^{15}N_2OPD$ measurements correlated positively with the same soil properties (R = 0.64–0.92, $p < 0.01$; Supplementary Table S1), whereas no correlation was found with net $N_2O$ flux measured by the GFSC method.

## Discussion

Both the $^{15}N_2OPD$ and GFSC methods have been proposed to be able to measure gross $N_2O$ production and consumption in soils[9,10]. The comparable net $N_2O$ fluxes determined by these methods (Fig. 1c) suggest that both methods can yield similar results in terms of the net effect of concurrently occurring production and consumption of $N_2O$. However, the measured gross $N_2O$ production and consumption rates (Fig. 1a,b), and thus the ratios of net $N_2O$ flux to gross $N_2O$ production (Fig. 1d), differed between the two methods. Hence, we reject our hypothesis that $^{15}N_2OPD$ technique and GFSC method yield comparable estimates of gross $N_2O$ fluxes.

When using the $^{15}N_2OPD$ technique, gross $N_2O$ production is determined from the dilution of $^{15}N_2O$ label by $^{14}N_2O$ produced in the soil[10]. An implicit assumption of this approach is that the headspace-labelled $^{15}N_2O$ that diffuses into the soil results in a homogeneous mixture of $^{15}N_2O$ with soil-derived $N_2O$ in the soil air-filled pores, which also imply that these pores must be interconnected to the soil surface for homogenous mixing to occur. Our conservative calculations of diffusive transport of $^{15}N_2O$ into interconnected soil air-filled pores suggest that $^{15}N_2O$ must have diffused into these pores and back to the headspace within 0.5 h. However, there may be two situations when gross $N_2O$ production and consumption will be underestimated by this method: 1) produced $N_2O$

| Soil characteristics | Grassland | Cropland | Beech forest | Pine forest |
|---|---|---|---|---|
| Water-filled pore space (%) | 79 ± 1 a | 57 ± 2 ab | 70 ± 14 ab | 25 ± 1 b |
| $NH_4^+$ (mg N $kg^{-1}$) | 4.34 ± 0.97 a | 0.66 ± 0.12 b | 2.35 ± 0.37 a | 1.30 ± 0.18 ab |
| $NO_3^-$ (mg N $kg^{-1}$) | 1.00 ± 0.14 b | 5.42 ± 0.60 a | 4.17 ± 2.14 ab | 0.71 ± 0.38 b |
| Microbial C (g C $kg^{-1}$) | 3.26 ± 0.13 a | 0.76 ± 0.03 c | 2.68 ± 0.24 ab | 1.72 ± 0.10 bc |
| Microbial N (mg N $kg^{-1}$) | 211.02 ± 4.84 a | 69.22 ± 0.90 c | 160.90 ± 11.35 ab | 98.70 ± 5.37 bc |
| Denitrification enzyme activity (g N $kg^{-1}$ $h^{-1}$) | 5.16 ± 0.64 a | 0.21 ± 0.07 bc | 0.83 ± 0.17 ab | 0.00 ± 0.00 c |

**Table 2. Soil physical and biochemical characteristics in the top 5 cm, determined from the soil cores immediately after the measurement of gross $N_2O$ fluxes.** Means ± s.e. (n = 4) within each row followed by different letter indicate significant differences among sites (one-way ANOVA with Fisher's LSD test at $p \leq 0.05$ or Kruskal-Wallis ANOVA with multiple comparisons of mean ranks at $p \leq 0.05$.

is immediately consumed within denitrifier cells[3], and 2) produced $N_2O$ diffuses out of denitrifier cells and is consumed by other microorganisms, which may have $N_2O$ reductase but cannot act on the preceding substrates of the denitrification pathway[18], without being mixed first with the $^{15}N_2O$ label during the 3-hour measurement period. Both situations can occur in anaerobic microsites, which here we infer to microsites saturated with water, isolated pores filled with or enclosed by water forming a diffusion barrier, and water-entrapped $N_2O$ as expounded by Clough *et al.*[14]. If these situations happen, disparity between $^{15}N_2O$PD and GFSC measurements would be large in a fine-textured soil with high water content whereas they would be comparable in a coarse-textured soil with low water content. The fact that our results showed the large differences between the $^{15}N_2O$PD and GFSC measurements in the silty loam soil of grassland with high WFPS and they were particularly comparable in the sandy soil of pine forest with low WFPS (Fig. 1a,b; Table 2) suggest that the $^{15}N_2O$PD technique was not able to quantify gross $N_2O$ production in these above-mentioned two situations. With the GFSC method, gross $N_2O$ production is measured as the sum of emitted $N_2O$ and $N_2$, and thus those immediately consumed $N_2O$ to $N_2$ within denitrifier cells and between different microorganims in anaerobic microsites are included in this measurement.

We summarize our results into a conceptual model in order to illustrate two decoupled pathways of $N_2O$ production and consumption in soil (Fig. 2). In the first pathway, $N_2O$ is produced in anaerobic microsites and reduced immediately to $N_2$ without first mixing with the $^{15}N_2O$ label. Based on our results, only the GFSC method but not the $^{15}N_2O$PD technique was able to quantify this pathway. The second pathway covers the soil-derived $N_2O$ that diffuses into the interconnected soil air-filled pores and mixes with the $^{15}N_2O$ label, which was captured by the $^{15}N_2O$PD technique. Even if the $N_2O$ that has moved into the soil air-filled pores is being consumed during its diffusion towards the soil-atmosphere interface[4], as long as the produced $N_2O$ mixes with the $^{15}N_2O$ label this can be included in the $^{15}N_2O$PD calculations of gross $N_2O$ production. It is clear that both $^{15}N_2O$PD and GFSC methods yield complementary important information, and thus a differentiation in the use of terminologies is needed. Since the $^{15}N_2O$PD technique reflects the $N_2O$ dynamics in the gas phase of the soils and its exchange with the atmosphere, we propose to use the terms 'gross $N_2O$ emission' and 'gross $N_2O$ uptake' to denote the gross $N_2O$ fluxes in interconnected soil air-filled pores measured by this method. Since the GFSC method measures gross $N_2O$ fluxes not only in interconnected soil air-filled pores but also in anaerobic microsites, we propose that the terms 'gross $N_2O$ production' and 'gross $N_2O$ consumption' be used (Fig. 2). Below we will use these proposed terminologies to distinguish between the processes measured by these two methods.

It is important to point out that the $^{15}N_2O$PD technique is able to yield information on gross $N_2O$ uptake from the atmosphere to the soil. For years there has been a discussion on the importance of $N_2O$ uptake in the soil from the atmosphere and substantial progress has been hampered because until now only the net $N_2O$ fluxes on the soil surface can be routinely measured with inexpensive static chamber method. With the $^{15}N_2O$PD technique, we now have an operational approach that can be used for field measurements and can separate the net $N_2O$ fluxes across the soil-atmosphere interface into gross $N_2O$ emission and gross $N_2O$ uptake. It is a significant advancement since this technique will allow us to investigate the factors that control $N_2O$ uptake by soils under actual field conditions, which is a commonly unquantified sink of ecosystem N budgets.

Moreover, our results contrast to the notion that substantial $N_2O$ uptake only happens in soils with net negative $N_2O$ flux. This was shown by the larger gross $N_2O$ uptake (measured by $^{15}N_2O$PD technique) in the grassland that had larger net $N_2O$ emissions than in the pine forest that had a net negative $N_2O$ flux (Fig. 1b,c). The positive correlations of gross $N_2O$ uptake with soil biochemical characteristics (Supplementary Table S1) suggest that high gross $N_2O$ uptake occurs in soils with high microbial activity and high substrate availability (Table 2). The ratios of net to gross $N_2O$ emissions (66–79% in grassland, cropland and beech forest; Fig. 1d) were similar to the values reported by Yang *et al.*[10] and Yang and Silver[12] from managed grassland and cropland in California (net to gross $N_2O$ emission ratio of 68–70%). These generally comparable ratios may open the possibility of making estimates of gross $N_2O$ emissions and uptake based on measured net $N_2O$ emissions.

The large fraction of gross $N_2O$ production that was consumed to $N_2$ (measured by GFSC method) suggests that gross $N_2O$ production and consumption were closely coupled, which is in line with our aforementioned deduction (i.e. most $N_2O$ was immediately reduced to $N_2$ in anaerobic microsites). Hence, the similar correlations found for gross $N_2O$ production and consumption with soil biochemical characteristics (Supplementary Table S1) as those found for gross $N_2O$ emission and uptake (measured by $^{15}N_2O$PD technique) suggests that these gross $N_2O$ fluxes were regulated by the same process, denitrification[4].

Our findings show that whereas the $^{15}N_2O$PD technique is a valuable tool to separate net $N_2O$ flux across the soil-atmosphere interface into gross $N_2O$ emission and uptake, it did not allow measuring a large part of gross

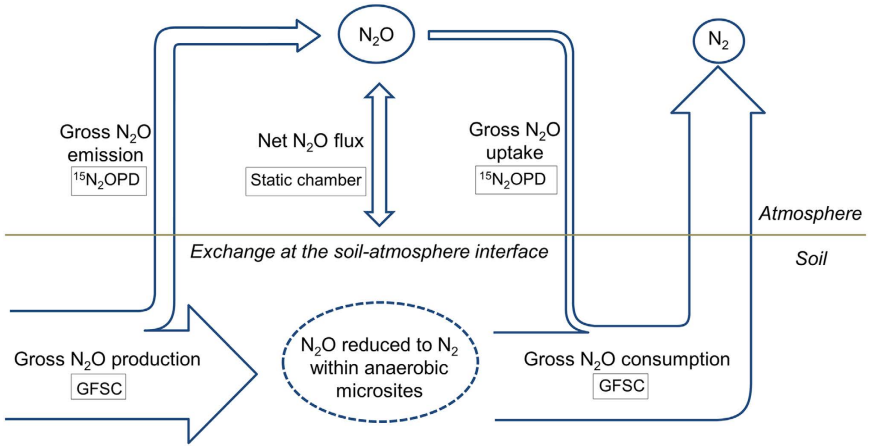


**Figure 2. Conceptual diagram of gross $N_2O$ fluxes.** Gross $N_2O$ emission and gross $N_2O$ uptake, measured by $^{15}N_2O$ pool dilution ($^{15}N_2OPD$), which largely includes gas exchange in interconnected air-filled pores in the soil; gross $N_2O$ uptake = gross $N_2O$ emission – net $N_2O$ flux. Gross $N_2O$ production and gross $N_2O$ consumption, measured by gas-flow soil core (GFSC), which encompasses the soil air-filled pores as well as anaerobic microsites (e.g. soil micro spots saturated with water, isolated pores filled with or enclosed by water, and water-entrapped $N_2O$); gross $N_2O$ consumption = $N_2$ emission, and gross $N_2O$ production = gross $N_2O$ consumption + net $N_2O$ flux.

$N_2O$ production and consumption in anaerobic microsites. In order to avoid misinterpretations of terminologies, we propose that the terms 'gross $N_2O$ emission and uptake' should be used for gross $N_2O$ fluxes measured with the $^{15}N_2OPD$ technique and 'gross $N_2O$ production and consumption' should be used for gross $N_2O$ fluxes measured with the GFSC method.

## Methods

**Study sites and soil sampling.** Soil samples were collected from four ecosystems: grassland, cropland, beech and pine forests, covering different vegetation, soil types and climatic conditions (Table 1). The montane grassland is manured 2–3 times a year and cut for hay three times a year[19]. The cropland is a conventional corn-winter wheat rotation. The unmanaged beech forest (*Fagus sylvatica)* is 163 years old[20], and the unmanaged Mediterranean pine forest (*Pinus pinaster*) is 52 years old[21].

At each site, we selected four sampling points as replicates with a minimum distance of 25 m from each other. At each replicate, eight intact soil cores (250 $cm^3$ each) were taken using stainless-steel cores (8 cm diameter, 5 cm height): four of which were used for the $^{15}N_2OPD$ measurement and the other four for the GFSC measurement. The $^{15}N_2OPD$ measurement was conducted concurrently with the GFSC measurement, such that the soil cores for these two methods were handled similarly in all aspects. Neither soil moisture nor substrate level was adjusted.

**$^{15}N_2O$ pool dilution.** Four intact soil cores were placed in an incubation glass (6.6 L volume), equipped with Luer-lock stopcock for gas sampling. Upon closure of the incubation vessel, we injected into the chamber headspace 7 mL of $^{15}N_2O$ label gas, containing 100 ppmv of 98% single labelled $^{15}N$-$N_2O$, 275 ppbv sulfurhexafluoride ($SF_6$, as a tracer for physical loss of $N_2O$) and the rest as synthetic air. This injected amount increased the $N_2O$ concentration in the headspace by approx. 106 ppbv $N_2O$ with 12.5 atom% $^{15}N$ enrichment and $SF_6$ concentration of 292 pptv. At 0.5, 1, 2, and 3 h following label gas injection, 100 mL and 12 mL gas samples were taken out and stored in pre-evacuated 100 mL glass bottles and 12 mL glass tubes (Exetainer; Labco Limited, Lampeter, UK), respectively, with rubber septa. The sampled air volume was then replaced with 112 mL of a gas mixture (80% helium and 20% oxygen) to maintain the headspace at atmospheric pressure and oxygen concentration, without altering the isotopic composition of the headspace $N_2O$. The dilution that this replacement caused was accounted for in the calculations. The 100 mL gas samples were used to analyze isotopic composition using an isotope ratio mass spectrometer (IRMS) (Finnigan Delta$^{plus}$ XP, Thermo Electron Corporation, Bremen, Germany). The 12 mL gas samples were used to measure $N_2O$ and $SF_6$ concentrations using a gas chromatograph equipped with an electron capture detector (GC 6000 Vega Series 2, Carlo Erba Instruments, Milan, Italy). The detection limit of the entire measurement set-up and instrument precision was $<0.9$ ppbv $N_2O$ $h^{-1}$.

We modeled the vertical diffusive transport of $^{15}N_2O$ label through the 5 cm long soil cores, using the diffusion equation $\frac{\partial C}{\partial t} = \frac{\partial^2 C}{\partial x^2}$ in which C, t and x denote concentration, time and path length, respectively[22]. The free-air $N_2O$ diffusion coefficient at 15 °C, 0.1582 cm $s^{-1}$, was used and adjusted for soil tortuosity based on the air-filled porosity[23], which was calculated using the measured bulk density and gravimetric moisture contents. Our most conservative calculations, using the lowest air-filled porosity and assuming an impervious boundary condition at bottom of the soil cores, showed that the $^{15}N_2O$ label had diffused into the 5 cm long soil cores and back to the headspace within 0.5 h. Thus, our sampling interval during the 3-hour measurement period was sufficient to allow mixing of the label gas with the soil-derived $N_2O$ in interconnected air-filled pores and to quantify the changes in $N_2O$ concentrations and $^{15}N_2O$ enrichments in the headspace.

Gross $N_2O$ emission rate was calculated using the following equations modified from Yang *et al.*[10]:

$$[^{14}N_2O]_t = \frac{F_{14} \times P}{(k_{14} + k_l)} - \left\{ \frac{F_{14} \times P}{(k_{14} + k_l)} - [^{14}N_2O]_0 \right\} \times \exp\{-(k_{14} + k_l) \times (t - t_0)\} \quad (1)$$

$$[^{15}N_2O]_t = \frac{F_{15} \times P}{(k_{15} + k_l)} - \left\{ \frac{F_{15} \times P}{(k_{15} + k_l)} - [^{15}N_2O]_0 \right\} \times \exp\{-(k_{15} + k_l) \times (t - t_0)\} \quad (2)$$

where $[^{14}N_2O]_t$ is the concentration of $^{14}N_2O$ at time t, calculated as the product of $N_2O$ concentration and $^{14}N$-$N_2O$ atom%; $[^{15}N_2O]_t$ is the concentration of $^{15}N_2O$, calculated as the product of $N_2O$ concentration and $^{15}N$-$N_2O$ atom% excess, assuming that the $^{15}N$ isotopic composition of background $N_2O$ is 0.3688 atom%[10]; t represents the time of gas sampling from the headspace; $F_{14}$ represents the $^{14}N_2O$ mole fraction (0.997) and $F_{15}$ represents the $^{15}N_2O$ mole fraction (0.003) of emitted $N_2O$; $k_{14}$ and $k_{15}$ represent the first-order rate constants of $^{14}N_2O$ and $^{15}N_2O$ reduction to $N_2$, respectively, calculated based on the fractionation factor ($\alpha = k_{15}/k_{14}$) that has an average value of $0.9924 \pm 0.0036$ in literature[10]; $k_l$ represents the first-order rate constant for loss of inert transport tracer, $SF_6$; P is gross $N_2O$ emission rate. The $k_{14}$ and $k_{15}$ represent the biological loss, and $k_l$ represents the physical loss. Since the changes of their concentrations in the headspace are simultaneously affected by biological consumption and physical loss, we used the sum of these constants ($k_{14} + k_l$ or $k_{15} + k_l$) in the above equations.

We estimated the parameters for P and $k_{15}$ by simultaneously fitting the measured $[^{14}N_2O]_t$ and $[^{15}N_2O]_t$ with equation (1) and (2). The best fit of $[^{14}N_2O]_t$ and $[^{15}N_2O]_t$ was found using the least square approach and minimizing the following error function:

$$E = \sum_{t=1}^{n} \frac{(Y_{predicted}(t) - Y_{observed}(t))^2}{SD} + \sum_{t=1}^{n} \frac{(Z_{predicted}(t) - Z_{observed}(t))^2}{SD} \quad (3)$$

where E is minimal weighted error (E); Y, Z and n indicate $^{14}N_2O$, $^{15}N_2O$ concentrations, and the number of measurements, respectively; SD refers to the standard deviation of the observed concentrations over the course of measurements[24,25]. Equation (3) was minimized using the 'fminsearchbnd' function in MATLAB (MathWorks, Version R2011b, USA). Gross $N_2O$ uptake was calculated as the difference between gross $N_2O$ emission and net $N_2O$ flux[10].

**Gas-flow soil core.** The GFSC method is a fully automated, direct and sensitive quantification of the change of $N_2O$ and $N_2$ concentrations in the headspace above the soil cores. The soil air of the four soil cores and the headspace of the incubation vessel were completely replaced by a gas mixture consisting of 20% $O_2$ (purity grade of 5.5), 80% He (purity grade of 5.0), $N_2O$ (400 ppbv) and $N_2$ (25 ppmv). This complete exchange was done by automated repeated cycles of evacuation and gas purging, achieved through a built-in purging system in an extremely air-tight chamber that is connected directly to a gas chromatograph (Shimadzu GC-17A, Shimadzu, Munich, Germany)[17,26–28]. Eighteen hours of evacuation-purging cycles ensure a complete removal of the background atmospheric air[27], after which the headspace and tubing connections to the gas chromatograph were further purged for three hours. Subsequently, the system switched to a static chamber mode, and the headspace air of the incubation vessel was analyzed hourly over four hours through a directly connected gas chromatograph with an electron capture detector for $N_2O$ analysis and a pulse discharge He ionization detector (Vici AG, Schenkon, Switzerland) for $N_2$ analysis[26]. To sample the headspace, a slight overpressure was created by injecting 40 mL of the He-based gas mixture to the headspace, directing headspace air to the sampling loops[26]. The dilution of this non-intrusive overpressure sampling technique was accounted for in the calculation of $N_2O$ and $N_2$ concentrations[26]. In order to achieve the best possible tightness of the incubation system against intrusion of atmospheric $N_2$, all tubing connections, valves as well as the entire incubation vessel were placed under water. Before starting the $N_2O$ and $N_2$ measurements, the air-tightness of the system was always checked with an empty incubation vessel, which was connected in parallel with the measuring vessel. Based on the sensitivity and repeatability of the gas chromatograph measurements, the detection limits were $<0.03$ ppmv $h^{-1}$ for $N_2$ and $<0.45$ ppbv $h^{-1}$ for $N_2O$. The measured $N_2$ flux from the soil equals to gross $N_2O$ consumption whereas the sum of $N_2$ and $N_2O$ fluxes equals to gross $N_2O$ production[17,26–28].

**Soil controlling factors.** Soil water content (one-day oven-drying at 105 °C and expressed as WFPS using 2.65 g $cm^{-3}$ as particle density and the measured bulk density; Table 1), $NH_4^+$ and $NO_3^-$ concentrations (0.5 M $K_2SO_4$ extraction), and microbial biomass C and N ($CHCl_3$ fumigation-extraction) were determined from the soil cores immediately after the gas measurements. $NH_4^+$ and $NO_3^-$ concentrations in the soil extract were determined using continuous flow autoanalyzer (Skalar Scan plus system, Skalar Analytical B.V., Breda, Netherlands). Microbial biomass C and N were determined as the difference in 0.5 M $K_2SO_4$-extractable organic C and N (analyzed using persulfate oxidation with an infrared detector; Multi N/C 3100 TOC/TNb-Analysator, Analytik Jena, Jena, Germany) between the fumigated and unfumigated soils divided by $k_{EC} = 0.45$ and $k_{EN} = 0.68$[29]. DEA was determined from the $N_2O$ produced during an anaerobic incubation with glucose and $NO_3^-$ added in excess and acetylene inhibited $N_2O$ reduction of to $N_2$[30].

**Statistical analysis.** The above soil properties, determined separately from the soil cores used for $^{15}N_2OPD$ and GFSC measurements, did not differ between these two measurements ($p > 0.05$; paired t test); thus, the values from the two measurements were averaged to represent a replicate sampling point. Data sets were first tested for

normal distribution (Shapiro-Wilk's test) and equality of variance (Levene's test). We used log-transformation for variables with non-normal distributions or unequal variances and assessed the differences in gross $N_2O$ fluxes and soil properties among sites using one-way analysis of variance (ANOVA) with Fisher's least significant difference test. When none of the data transformations were able to attain normal distribution and equality of variance, differences among sites were tested using the Kruskal-Wallis ANOVA with multiple comparisons test. The differences in gross and net $N_2O$ fluxes between the $^{15}N_2OPD$ and GFSC methods for each site were assessed using the paired t test. Relationships of gross $N_2O$ fluxes with soil properties were assessed using spearman rank correlation test. Statistical significance was set at $p \leq 0.05$. Statistical analyses were conducted using SPSS (SPSS, Chicago, Illinois, USA).

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## Author Contributions

M.D.C., E.V., M.D., B.W. and K.B.-B. designed the study; Y.W. and Z.C. carried out the measurements and analyzed data; B.W. modeled the diffusive transport of $^{15}N_2O$ label in soil; A.C. and Y.W. solved the $^{15}N_2OPD$ equations in MATLAB and experimentally tested them; G.W., Z.C., B.W., R.K., M.D. and K.B.-B. established the GFSC method; M.D. and Z.C. conceptualized Fig. 2; M.D.C., Y.W. and E.V. wrote most parts of the manuscript; all authors reviewed and rewrote parts of the manuscript.

## Additional Information

**Supplementary information** accompanies this paper at http://www.nature.com/srep

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