



*Research article*

## Nitrous oxide emissions from trees planted on a closed landfill site

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**Abstract:** Trees growing in natural and managed environments have the capacity to act as conduits for the transport of greenhouse gases produced belowground to the atmosphere. Nitrous oxide (N<sub>2</sub>O) emissions have been observed from tree stems in natural ecosystems but have not yet been measured in the context of forested former landfill sites. This research gap was addressed by an investigation quantifying stem and soil N<sub>2</sub>O emissions from a closed UK landfill and a comparable natural site. Measurements were made by using flux chambers and gas chromatography over a four-month period. Analyses showed that the average N<sub>2</sub>O stem fluxes from the landfill and non-landfill sites were  $0.63 \pm 0.06 \mu\text{g m}^{-2} \text{h}^{-1}$  and  $0.26 \pm 0.05 \mu\text{g m}^{-2} \text{h}^{-1}$ , respectively. The former landfill site showed seasonal patterns in N<sub>2</sub>O stem emissions and decreasing N<sub>2</sub>O fluxes with increased stem sampling position above the forest floor. Tree stem emissions accounted for 1% of the total landfill N<sub>2</sub>O surface flux, which is lower than the contribution of stem fluxes to the total surface flux in dry and flooded boreal forests.

**Keywords:** landfill; tree stem N<sub>2</sub>O; nitrogen cycle; spatial variability; temporal variability; GHG emissions.

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### 1. Introduction

N<sub>2</sub>O is a potent greenhouse gas (GHG) with an estimated radiative forcing of  $0.17 \text{ W m}^{-2}$  and a global warming potential of 298 over a 100-year timescale [1,2]. N<sub>2</sub>O is also a contributor to atmospheric NO<sub>x</sub>; approximately 10% of N<sub>2</sub>O that reaches the stratosphere is broken down via photolysis to NO<sub>x</sub>, which then catalytically destroys stratospheric O<sub>3</sub> [3,4]. Identifying and quantifying

the global sources and sinks of N<sub>2</sub>O is important for developing climate change mitigation strategies and reducing the rate of stratospheric O<sub>3</sub> depletion. N<sub>2</sub>O is produced in soils and oceans via nitrification and denitrification [5,6]. In anaerobic soils, N<sub>2</sub>O is produced as an intermediate step during denitrification, where nitrous oxides are reduced to form N<sub>2</sub> [7]. In aerobic soils, N<sub>2</sub>O is produced via nitrification, whereby NH<sub>3</sub> and NH<sub>4</sub><sup>+</sup> are converted to NO<sub>3</sub><sup>-</sup>, and N<sub>2</sub>O is produced as a by-product [7]. Terrestrial ecosystems account for c.33% of the total (natural and anthropogenic) global N<sub>2</sub>O emissions [2].

Trees growing in upland and wetland environments can provide a pathway for N<sub>2</sub>O produced belowground to the atmosphere [6,8,9]. N<sub>2</sub>O is transported through tree stems via absorption in the root zone and then diffusion through the xylem. N<sub>2</sub>O is emitted from tree stem surfaces after diffusing across the cambium and meristem to the bark, where it is released via lenticels [6]. Tree-mediated emissions can account for up to 10% of the total N<sub>2</sub>O ecosystem flux in upland environments, and up to 18% in wetlands [8–10]; however, stem N<sub>2</sub>O emissions have not been quantified in managed environments.

The remediation of landfill sites after closure often includes afforestation because it increases carbon sequestration, reduces water percolation into waste and improves the aesthetic appeal [11]. Trees growing on closed landfill sites emit methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>) [12,13]; however, their ability to emit N<sub>2</sub>O has not yet been investigated. N<sub>2</sub>O emissions from landfill sites can result from nitrification or denitrification, particularly when alternating aerobic and anaerobic zones exist within the waste [14]. N<sub>2</sub>O can also be formed in aerobic cover soils via methanotrophic nitrification, as methanotrophs co-oxidize CH<sub>4</sub> and ammonia [5,15]. Landfill surface fluxes are highly variable (0.05 and 81.9 mg m<sup>-2</sup> h<sup>-1</sup>), with total emissions from landfill and wastewater of c.0.3 Tg N<sub>2</sub>O yr<sup>-1</sup> [2,14,16].

This study aimed to develop an understanding of GHG emissions from trees on closed landfill sites by quantifying N<sub>2</sub>O fluxes for the first time. A four-month field campaign was designed to investigate spatial and temporal patterns and quantify the contribution of trees to total landfill surface N<sub>2</sub>O fluxes. The hypotheses for the study were as follows:

1. Trees growing on closed landfill sites will emit more N<sub>2</sub>O than trees growing in a natural comparable woodland;
2. N<sub>2</sub>O stem fluxes will be higher in the summer months;
3. N<sub>2</sub>O emissions decrease with height of the tree trunks (above the ground).

## 2. Materials and methods

The study sites, sampled trees and their characteristic measurements used in this investigation have previously been described by Fraser-McDonald et al. [12]. Sampling was carried out at a closed landfill and nearby non-landfill area. Waste was accepted at the landfill site from 1964 to 1998 before it was capped (clay of 1-m minimum depth; permeability: 10<sup>-7</sup> m s<sup>-1</sup>) and covered with 2 m of topsoil. Gas and leachate control systems were installed, and a 1.22 ha woodland was created on the site in 2004. Trees were planted on the non-landfill site to create secondary woodland in 2003. The tree species sampled at both sites were *Betula pendula*, *Fraxinus excelsior* and *Prunus avium*. Soils at both sites were loamy clay, with underlying sedimentary bedrock and mudstone, and the mean annual temperature and rainfall were 14.1 °C and 565.5 mm, respectively [17]. Tree and soil measurements were made at both sites between April and July 2021. Monthly measurements were obtained from 15

trees and 15 soil locations over a 2-day period each month (between 8:00 and 17:00), with sampling at 30, 90 and 150 cm stem heights (from ground level).

Semi-rigid flux chambers (30 × 15 × 3 cm) constructed from clear polycarbonate and closed-cell silicone foam strips were fastened to trees with ratchet straps and sealed with airtight putty [12]. Soil flux measurements were taken by using rigid cylindrical chambers that were inserted into the soil (rigid polyvinyl chloride cylinders with a radius and depth of 15 cm) [12]. Each chamber was fitted with a lid prior to samples being taken. Tree chambers and soil chamber lids were fitted with rubber septa to allow gas samples to be obtained (Suba-Seal, Sigma-Aldrich). Ten-ml air samples were taken via the septa by using a luer lock syringe and a side port stainless steel needle (to minimize septum coring) (Sigma-Aldrich). Each sample was ejected into a pre-evacuated 5.9 ml flat-bottomed vial (Extetainer, Labco). Vials were over-pressured to minimize the effect of any leakage. Samples were taken from soil and tree chambers immediately after closure, and then again after 20, 40 and 60 minutes.

N<sub>2</sub>O gas fluxes were calculated by using the ideal gas equation and standardized for temperature and pressure [12]. Three tree and soil locations per site visit were selected prior to the field campaign to test for flux linearity. Each sample (0, 20, 40 and 60 minutes) was analyzed before regression lines were determined, and the associated R<sup>2</sup> values were calculated. Once the linearity of fluxes was established, the remaining fluxes for each monthly site visit were calculated by using only the 0- and 60-minute samples.

Samples taken from the stem and soil chambers were analyzed by using gas chromatography. An Agilent 7890A gas chromatograph fitted with a micro-electron capture detector (μECD) and autosampler was used. Full details of this analysis are available in the Supplementary Methods.

Vial leakage tests were carried out to ensure the accuracy of stored samples over time. Vials were filled with a known N<sub>2</sub>O standard (1 ppm with ± 2% mixture accuracy) and stored for the same period of time as field samples from each month (April, May, June and July; three test vials per month). Regression analysis showed that the N<sub>2</sub>O concentration decreased over time; therefore, percentage corrections were applied to vial concentrations based on storage time (see Supplementary Methods for details).

Graphs were created in Origin (version 2020) and statistical tests were carried out using R (3.5.1) and SPSS (24). Parametric and non-parametric tests were employed to determine significant differences. Full details of the statistical tests used are detailed in the Tables S2 and S4. The methods described by Fraser-McDonald et al. [12] were used to scale up N<sub>2</sub>O stem and soil fluxes.

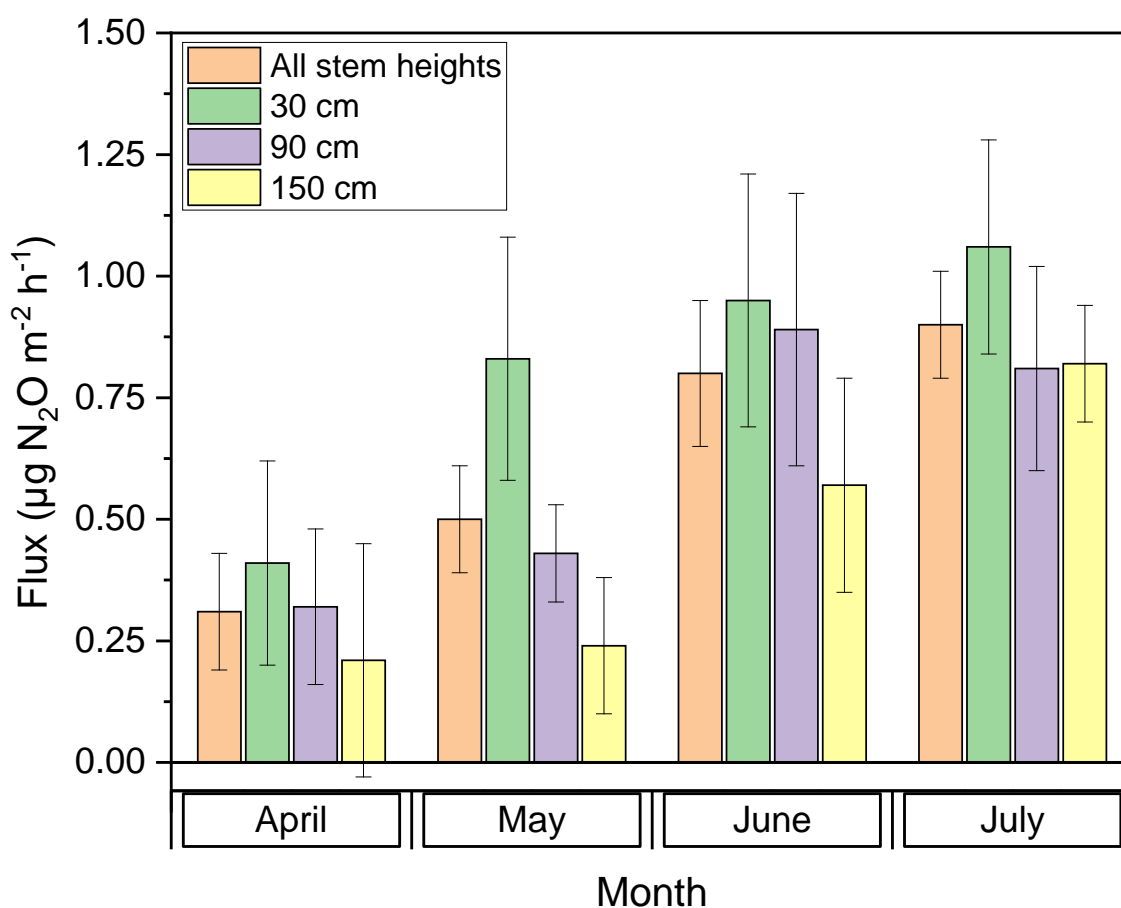
### 3. Results and discussion

Average N<sub>2</sub>O tree stem emissions from all measurement heights at the closed landfill site during the measurement period from April to July 2021 were  $0.63 \pm 0.06 \mu\text{g m}^{-2} \text{h}^{-1}$ . Mean soil N<sub>2</sub>O fluxes from the former landfill site were  $25.5 \pm 3.7 \mu\text{g m}^{-2} \text{h}^{-1}$ . Average N<sub>2</sub>O stem (all measurement heights) and soil fluxes from the comparable natural woodland were  $0.26 \pm 0.05 \mu\text{g m}^{-2} \text{h}^{-1}$  and  $3.53 \pm 0.58 \mu\text{g m}^{-2} \text{h}^{-1}$ , respectively.

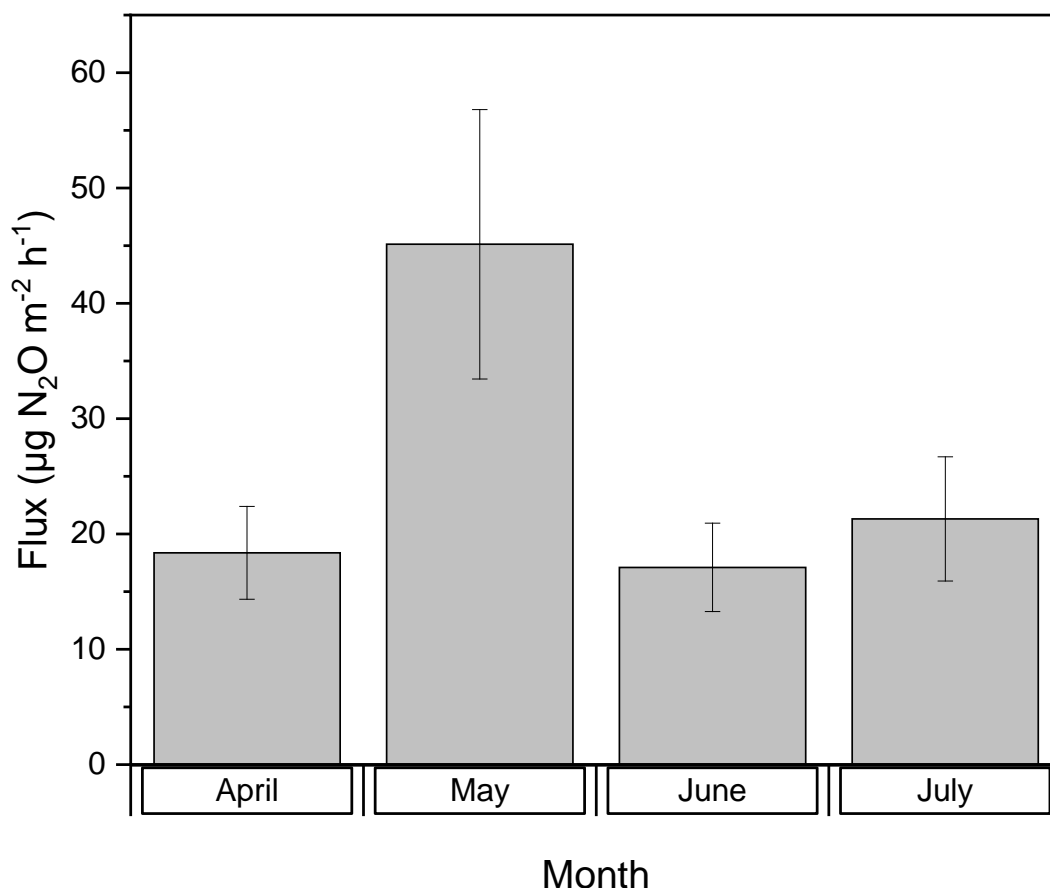
#### 3.1. Temporal variations in N<sub>2</sub>O fluxes

Average monthly N<sub>2</sub>O fluxes from tree stems on the former landfill site varied significantly (at all stem measurement heights) ( $P < 0.01$ ). N<sub>2</sub>O fluxes generally increased from April to July 2021 at all measurement heights, except for the 90 cm measurement height between June and July, where they

were statistically the same ( $P > 0.05$ ) (Figure 1). Average tree stem  $\text{N}_2\text{O}$  fluxes were *c.*2 times greater in the summer months (June and July) than in the spring (April and May), with values of  $0.85 \pm 0.09 \mu\text{g m}^{-2} \text{h}^{-1}$  in the summer and  $0.40 \pm 0.08 \mu\text{g m}^{-2} \text{h}^{-1}$  in the spring (Table S3). The observed trend of increasing tree stem emissions from April through to July at all stem heights concurs with a previous study in which  $\text{N}_2\text{O}$  emissions from tree stems in a boreal forest were higher in the summer months [9]. The pattern of increasing stem  $\text{N}_2\text{O}$  emissions throughout the measurement period is likely due to the combined effect of variations in air temperature and soil moisture. Variations in stem fluxes at 30 cm correspond with rainfall patterns, as average UK rainfall was low in April 2021, but higher in May and July 2021 (Table S6). Additionally, measurements were taken from the former landfill site within 3 days of rainfall in May and July, but not in April and June 2021, and, on average, soil moisture was higher in May than in other months (Table S6). Relatively high rainfall in May and July would increase the soil water content, likely leading to higher rates of denitrification as  $\text{O}_2$  becomes more limited [18].



**Figure 1.** Mean stem  $\text{N}_2\text{O}$  fluxes ( $\mu\text{g m}^{-2} \text{h}^{-1}$ ) for each month at each measurement height from the ground and average monthly  $\text{N}_2\text{O}$  fluxes for all stem heights combined; error bars show the standard error.



**Figure 2.** Mean soil N<sub>2</sub>O fluxes (µg m<sup>-2</sup> h<sup>-1</sup>) for each month; error bars show the standard error.

Soil N<sub>2</sub>O fluxes at the landfill site also varied significantly on a monthly basis ( $P < 0.01$ ) (Figure 2). Soil N<sub>2</sub>O emissions in May 2021 were significantly higher than those in April ( $P < 0.01$ ), June ( $P < 0.01$ ) and July 2021 ( $P < 0.01$ ). Soil fluxes in July were also significantly higher than those in June ( $P < 0.05$ ). Higher soil N<sub>2</sub>O emissions from the landfill site in months with increased rainfall and soil moisture indicated that rainfall events resulted in higher rates of denitrification. This agrees with results from previous investigations on closed landfills, where N<sub>2</sub>O fluxes declined with lower soil moisture due to the effect on microbial populations in cover soils [5].

Increased air temperatures in summer were expected to cause a decrease in N<sub>2</sub>O stem flux rates, as N<sub>2</sub>O reductase activity increases with higher temperatures. This results in more N<sub>2</sub>O being converted to N<sub>2</sub> during complete denitrification [19]. However, stem N<sub>2</sub>O emissions in June and July (the warmest months) were higher than in April and May. Because the average air temperatures in June and July were lower than the optimum temperature for denitrification in soils (25–30 °C), the rate of denitrification may have increased, but not to the extent that complete denitrification to N<sub>2</sub> dominated [20]. In contrast, soil N<sub>2</sub>O fluxes were not higher in months with lower rainfall and increased temperature (June 2021, for example). This suggests that there may be an additional temperature-dependent source of N<sub>2</sub>O production contributing to emissions from tree stems that is not present in soils. For example, N<sub>2</sub>O can be formed in plant tissues under anoxic conditions or after exposure to UV light [21]. If tree roots enter anaerobic zones in the soil or the landfill cap, N<sub>2</sub>O may

be produced in mitochondria under anoxic conditions [22]. Measurements taken during this study were constrained by time and sample size; future research should build on this initial quantification of changing N<sub>2</sub>O emissions over time by evaluating seasonal trends across an entire year.

### 3.2. Spatial variations in N<sub>2</sub>O tree fluxes

#### 3.2.1. Gas flux variations with measurement height

Average landfill tree stem N<sub>2</sub>O fluxes (for all months in the measurement period) at 30, 90 and 150 cm measurement heights were  $0.81 \pm 0.12 \mu\text{g m}^{-2} \text{h}^{-1}$ ,  $0.61 \pm 0.10 \mu\text{g m}^{-2} \text{h}^{-1}$  and  $0.46 \pm 0.10 \mu\text{g m}^{-2} \text{h}^{-1}$ , respectively (Figure 3). There was a general trend of decreasing fluxes with increased stem height and a significant difference between N<sub>2</sub>O emissions at 30 and 150 cm ( $P < 0.05$ ). Further analysis showed the same pattern of lower stem N<sub>2</sub>O fluxes with increased measurement height from ground level in each month during the observation period (Figure 1; Table S3). N<sub>2</sub>O emissions are expected to decrease with increased stem height when emissions are being channeled from an underground source [23]. For example, average N<sub>2</sub>O emissions from adult trees after soil fertilization in a temperate upland ecosystem decreased linearly with increased stem height [6]. The observed trend at the landfill site of decreasing N<sub>2</sub>O fluxes with increasing stem height is likely due to the N<sub>2</sub>O concentration in plant tissues becoming lower with height and, therefore, N<sub>2</sub>O diffusion being reduced [6]. In addition, higher parts of a tree stem generally have lower rates of transpiration, which could also explain the lower N<sub>2</sub>O flux rates with increased stem height [24,25]. These results indicate that N<sub>2</sub>O emitted by the trees growing at the landfill site originated from a belowground source, although it is not certain whether this source is in the waste or the cover soil. Although different sources of N<sub>2</sub>O were considered, deriving the exact source was beyond the scope of this study; future research should endeavor to identify the source of N<sub>2</sub>O emissions from tree stems at closed landfill sites. Also, closed landfills with varying ages and management practices should be investigated, as more recently closed sites have been shown to emit more N<sub>2</sub>O from the soil surface [14].

#### 3.2.2. Spatial variations in N<sub>2</sub>O fluxes at the landfill site

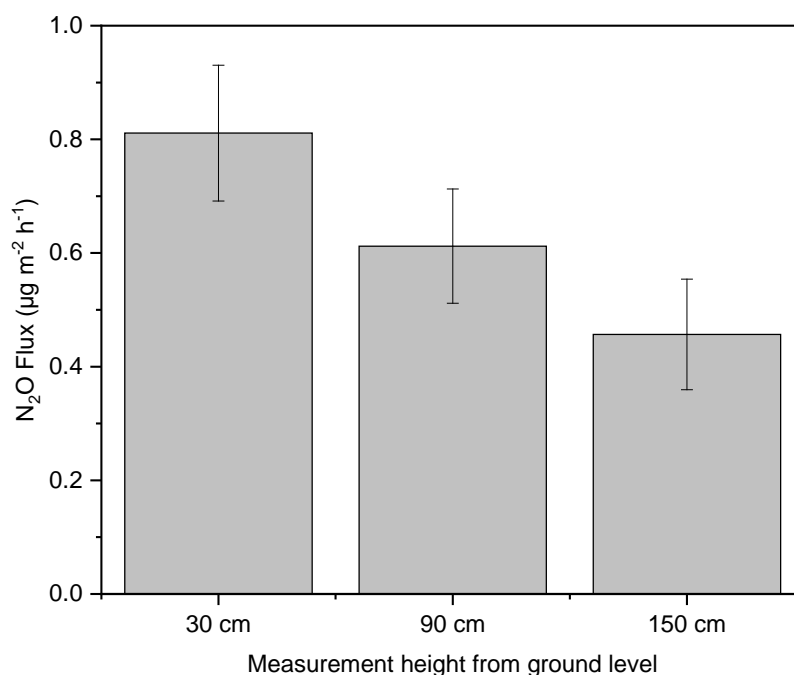
Spatial variations in stem and soil N<sub>2</sub>O fluxes from the closed landfill site (including data from all measurement heights and months) are shown in Figure 4. The highest average N<sub>2</sub>O stem fluxes were largely in the west and northwest, and the lowest average stem fluxes were in the east and southwest areas of the site. The largest N<sub>2</sub>O soil fluxes were observed on the eastern edge of the site and in relatively small areas in the southwest and northwest. The lowest average soil N<sub>2</sub>O fluxes were largely in the central area of the site. Unlike previously reported CH<sub>4</sub> emissions from the landfill site [12], none of the sampled locations consistently emitted substantially more N<sub>2</sub>O than others. This indicates that the source of N<sub>2</sub>O was more uniform across the site than the source of CH<sub>4</sub>.

### 3.3. Variations in N<sub>2</sub>O fluxes from landfill

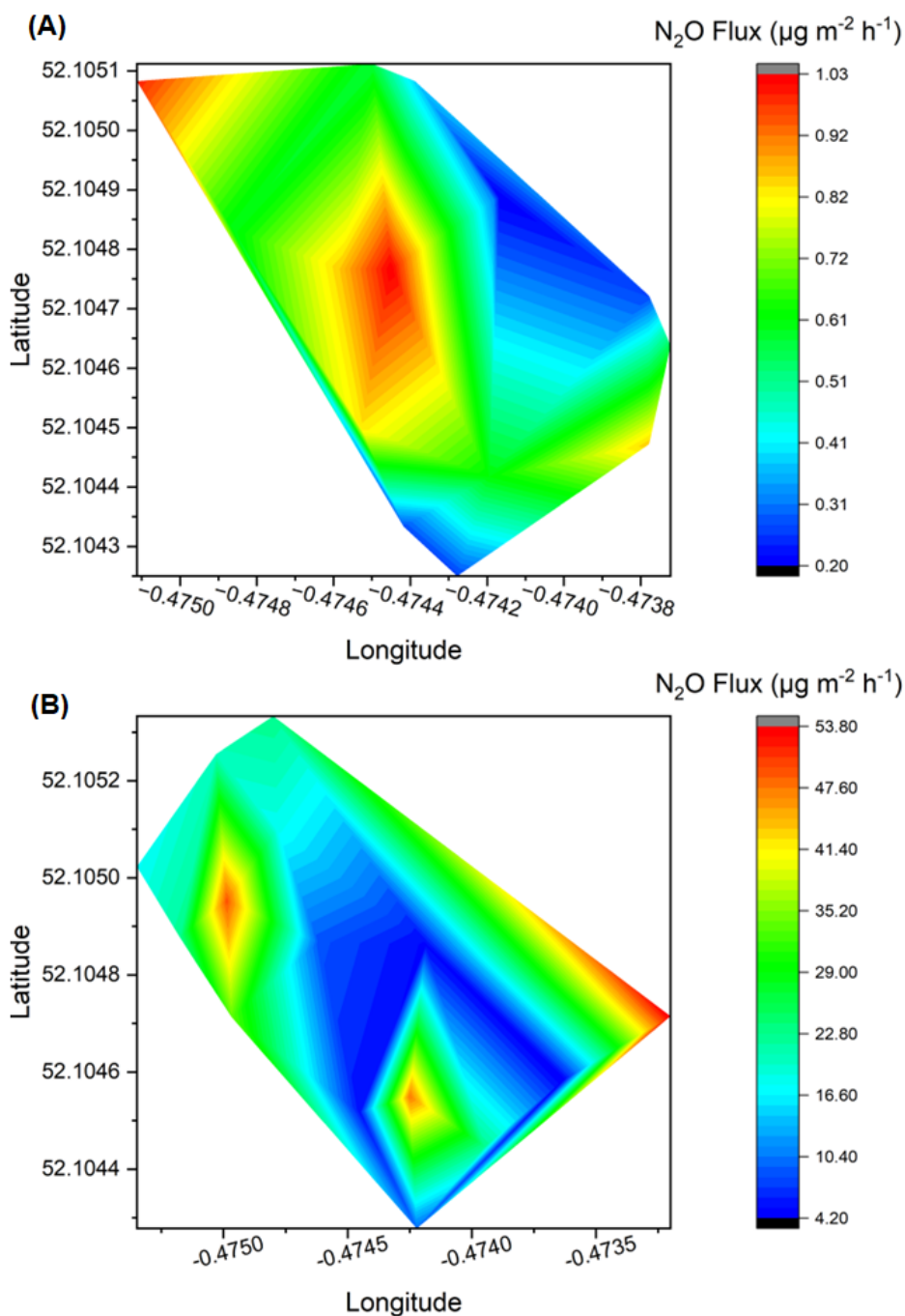
Observed average soil and stem fluxes indicated that the closed landfill site and comparable non-landfill area were net sources of N<sub>2</sub>O throughout the measurement period. Landfill soil N<sub>2</sub>O measurements ranged from 0.270 to 153.9  $\mu\text{g m}^{-2} \text{h}^{-1}$ , which is comparable to the lower end of the range of N<sub>2</sub>O fluxes previously measured from landfill surfaces with soil cover ( $-1.7$  to  $575 \mu\text{g m}^{-2} \text{h}^{-1}$ ) [16]. The highest soil N<sub>2</sub>O measurements from this investigation were of the same order of magnitude as a

site in Sweden with a cover comprised of ash, bark and sand and no gas extraction (fluxes ranged from  $-1.7$  to  $163 \mu\text{g m}^{-2} \text{h}^{-1}$ ) [16]. The relatively low fluxes from the investigated landfill are likely due to the age of the site (closure occurred in 1998), as well as factors such as soil moisture, soil temperature and the availability of mineral nitrogen (the effects of soil temperature and soil moisture are discussed further below) [14].  $\text{N}_2\text{O}$  fluxes from this site were also substantially lower than those from active sites and those with a cover of sewage sludge [14].

$\text{N}_2\text{O}$  fluxes from tree stems were significantly different between the closed landfill site and the comparable non-landfill location ( $P < 0.01$ ). Average stem  $\text{N}_2\text{O}$  emissions from the landfill ( $0.63 \pm 0.06 \mu\text{g m}^{-2} \text{h}^{-1}$ ) were higher than those from the non-landfill site ( $0.26 \pm 0.05 \mu\text{g m}^{-2} \text{h}^{-1}$ ) (Figure 5). There were also significant differences in the stem  $\text{N}_2\text{O}$  fluxes between the two sites when fluxes at each measurement height from the ground were considered individually.  $\text{N}_2\text{O}$  fluxes from trees at the landfill site were significantly larger than those at the non-landfill site at 30 and 90 cm measurement heights ( $P < 0.05$  and  $P < 0.01$ , respectively) (Table S7). There was a marginal difference in the stem  $\text{N}_2\text{O}$  fluxes between the two sites at the 150 cm measurement height ( $P = 0.071$ ). Average soil  $\text{N}_2\text{O}$  fluxes from the landfill and non-landfill sites during the measurement period were  $25.48 \pm 3.72 \mu\text{g m}^{-2} \text{h}^{-1}$  and  $3.53 \pm 0.58 \mu\text{g m}^{-2} \text{h}^{-1}$ , respectively. These differences may be due to factors such as soil moisture, soil temperature or nitrogen availability [14]. The effects of air temperature and soil moisture on tree stem  $\text{N}_2\text{O}$  fluxes was discussed above. The average stem  $\text{N}_2\text{O}$  flux for the landfill site was within the reported range of fluxes from trees in a non-flooded temperate woodland [9]. The range of stem flux values from the landfill site ( $-1.43$  to  $3.39 \mu\text{g m}^{-2} \text{h}^{-1}$ ) was smaller than that from a temperate woodland ( $-11.87$  to  $30.28 \mu\text{g m}^{-2} \text{h}^{-1}$ ), where variability was reported to be high [9]. This indicates that trees planted at closed landfill sites do not emit atypical levels of  $\text{N}_2\text{O}$  compared with previously published emissions from trees planted in natural woodlands.

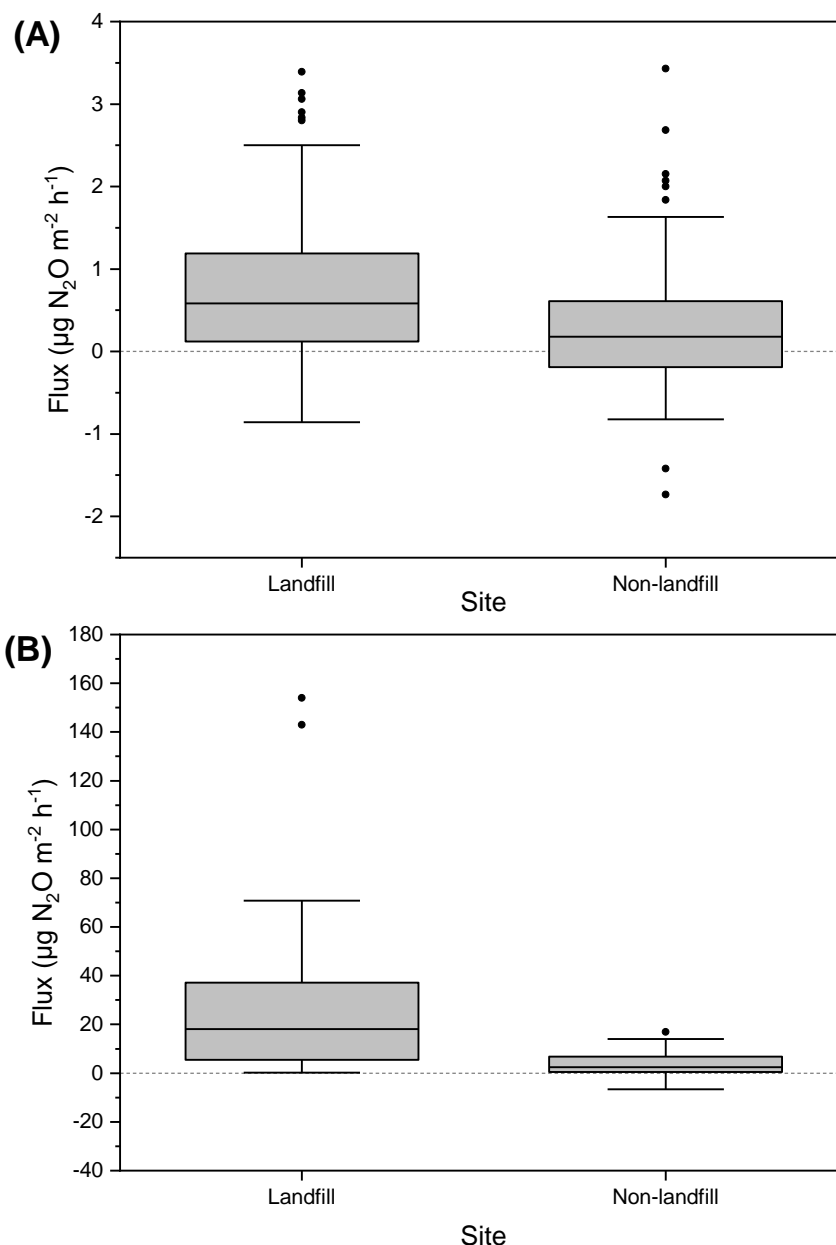


**Figure 3.** Mean  $\text{N}_2\text{O}$  fluxes ( $\mu\text{g m}^{-2} \text{h}^{-1}$ ) at each measurement height from the ground; error bars show the standard error.



**Figure 4.** Contour plots showing spatial variation in (A) stem  $\text{N}_2\text{O}$  fluxes and (B) soil  $\text{N}_2\text{O}$  fluxes at the landfill site (including data from all months and stem heights). Note different scales for each plot.





**Figure 5.** (A). Boxplot comparing tree stem N<sub>2</sub>O fluxes between the landfill and non-landfill site (all months and measurement heights). (B) Boxplot comparing soil N<sub>2</sub>O fluxes between the landfill and non-landfill site (all months). The middle line indicates the median value, and the whiskers are determined by the 5<sup>th</sup> and 95<sup>th</sup> percentiles. The dots represent outliers (below:  $Q_1 - 1.5 \text{ IQR}$  or above:  $Q_3 + 1.5 \text{ IQR}$ ).

Tree stem N<sub>2</sub>O emissions from this landfill site were substantially lower than previously reported CH<sub>4</sub> and CO<sub>2</sub> emissions [12]. When the 100-year global warming potentials (GWP<sub>100</sub>) were considered, the average N<sub>2</sub>O flux from this closed landfill was equivalent to 166.95 µg CO<sub>2</sub>e m<sup>-2</sup> h<sup>-1</sup>. This is lower than the average tree stem CH<sub>4</sub> flux for the same site (320 µg CO<sub>2</sub>e m<sup>-2</sup> h<sup>-1</sup>), and substantially lower than reported CO<sub>2</sub> stem fluxes (53.3 mg CO<sub>2</sub>e m<sup>-2</sup> h<sup>-1</sup>) [12]. The upscaled landfill N<sub>2</sub>O soil surface flux from the landfill site was 2723 g N<sub>2</sub>O yr<sup>-1</sup>, and the upscaled stem flux was 26 g N<sub>2</sub>O yr<sup>-1</sup>. The stem and soil emissions were equal to 7.7 and 811.5 kg CO<sub>2</sub>e yr<sup>-1</sup>, respectively. The tree

stem N<sub>2</sub>O emissions accounted for c.1% of the estimated total landfill surface flux. This agrees with findings from a mesocosm study in which tree stem emissions accounted for 1–3% of the total ecosystem N<sub>2</sub>O emissions [8]. These results indicate that excluding N<sub>2</sub>O fluxes from tree stems may result in an underestimation of the amount of N<sub>2</sub>O emitted from former landfills. However, estimates of the contribution of stem fluxes to the total surface N<sub>2</sub>O flux in dry and flooded boreal forests were higher, with values of 8% and 18%, respectively [10].

#### 4. Conclusions

This study has revealed that landfill sites with forested areas have the potential to be a net source of N<sub>2</sub>O, with tree stem fluxes accounting for 1% of the total landfill surface N<sub>2</sub>O emissions. Fluxes from the landfill site showed temporal variability, as N<sub>2</sub>O emissions in the June and July were higher than those in April and May, likely due to variations in temperature and soil moisture. Stem fluxes were lower with increased height from the ground, and this spatial variation indicated a belowground source of N<sub>2</sub>O. Findings presented here demonstrate that trees planted on a closed landfill site have the capacity to emit more N<sub>2</sub>O than a comparable non-landfill area. However, the contribution of stem N<sub>2</sub>O fluxes to the total surface flux on the former landfill was at a similar or lower magnitude than that of fluxes previously reported from natural forested ecosystems. There is a need for further investigation into the environmental factors that influence the magnitude of N<sub>2</sub>O fluxes from landfills and the source of these N<sub>2</sub>O emissions. Further measurements should also be taken to identify seasonal trends in N<sub>2</sub>O emissions over an entire year. This would aid in further explaining the variability in N<sub>2</sub>O fluxes and mitigating legacy emissions from closed landfill sites. These results indicate that trees growing on closed landfills have the capacity to emit N<sub>2</sub>O from a belowground source, and that including N<sub>2</sub>O released via tree stems in GHG budget assessments for former landfills may be beneficial.

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#### Conflict of interest

All authors declare no conflict of interest regarding the publication of this paper.

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