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The Effect of Cutting and Waterlogging on Plant-Related CO_2 and N_2O Fluxes Associated with the Invasive N-Fixing Species *Gunnera tinctoria*

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Abstract: The overall impact that plant invasions have on greenhouse gas emissions (GHG) by plant-mediated effects and how these interact with environmental and management factors is largely unknown. To address this, we report on the effects of leaf removal and waterlogging, either singularly or in combination, on the fluxes of CO_2 and N_2O associated with the invasive species *Gunnera tinctoria*. Both the removal of leaves with and without flooding resulted in higher CO_2 emissions due to reductions in photosynthesis. Whilst waterlogging alone was also associated with a reduction in photosynthesis, this was slower than the effect of leaf removal. Significant N_2O emissions were associated with intact plants, which increased immediately after leaf removal, or seven days after waterlogging with or without leaf removal. We found positive correlations between CO_2 and N_2O emissions and petiole and rhizome areas, indicating a size-dependent effect. Our results demonstrate that intact plants of *G. tinctoria* are a source of N_2O emissions, which is enhanced, albeit transiently, by the removal of leaves. Consequently, management interventions on invasive plant populations that involve the removal of above-ground material, or waterlogging, would not only reduce CO_2 uptake, but would further compromise the ecosystem GHG balance through enhanced N_2O emissions.

Keywords: leaf removal; invasive alien plants; greenhouse gas emissions; nitrous oxide; plant-mediated emissions; waterlogging

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1. Introduction

Plant invasions represent a major change in land-cover [1,2], often resulting in significant alterations in soil biogeochemical processes [3–5] and carbon and nitrogen stocks [6]. Although these changes could have a significant impact on ecosystem greenhouse gas (GHG) emissions, this remains largely unquantified and may be dependent on the specific GHG under investigation [7,8]. Plant invasions might, through their influence on water availability, labile C production, modifications in soil pH, and increased oxygenation of the rhizosphere [9,10], influence several C and N transforming reactions that could lead to alterations in GHG emissions. Nonetheless, the mechanism(s) by which invasive plant populations affect GHG emissions are still poorly understood.

There is also an increasing recognition that many plants including the well-known invasive species *Phragmites australis* (common reed) may enhance GHG emissions by providing a low resistance pathway for the diffusion of gases from the soil to the atmosphere [11–15]. Plants may also contribute directly to GHG emissions with high N_2O emissions, as reported in areas dominated by the macrophyte *Phalaris arundinacea* [16]. It has also been demonstrated that *Alnus glutinosa* trees were responsible for up to 64% of CH_4 emissions [17] and significant N_2O and CH_4 emissions were derived from stems and shoots of *Pinus sylvestris* [18]. Similarly, high emissions of both CH_4 and N_2O were found from the stems of trees grown under flooded conditions [19]. Importantly, in a recent review of the literature, it was suggested that the presence of vegetation can be responsible for up to 22% of annual CH_4 emissions globally [20].

While plant invasions may directly or indirectly facilitate GHG emissions, this may also be impacted by growth under waterlogged conditions. High water levels can have complex effects on soil biogeochemical and microbial processes that lead to modifications in soil GHG fluxes [21]. Whilst flooding-related increases in plant-mediated N_2O emissions may be dependent on nitrate levels [19,22], this could also be related to the increased availability of labile carbon due to the increased decomposition of plant roots [9,23] and/or the extent of rhizosphere oxidation [24]. On the other hand, management interventions that result in the removal of all or part of the vegetation could have quite different outcomes on soil GHG emissions [8]. If the plants simply act as a conduit for gaseous diffusion, the removal of above-ground plant parts could lead to a reduction in GHG emissions, although this may depend on whether all or some of the above-ground parts are removed. Conversely, if plants contribute directly to GHG production, the removal of above-ground plant parts could either reduce or enhance GHG emissions, depending on the mechanisms involved and the extent of gas exchange between the sources of production within plant tissues and the surrounding atmosphere.

In one of the first experiments on the effects of removing part of the standing vegetation (i.e., clipping), higher CH_4 emissions were associated with clipped *Carex* sp. (wetland sedge) plants [25]. It has also been demonstrated that the grazing of *P. australis* by geese increased CH_4 emissions with shoot removal, possibly enhancing methane transportation from the soil to the atmosphere [14]. Damage to vegetation through herbivory may also strongly influence the community structure of soil microorganisms, reducing the carbon stock capacity of ecosystems [26], with resultant modifications in GHG emissions [27]. Therefore, vegetation removal may affect ecosystem GHG balance, especially if applied in large areas to control invasive plant populations, something that remains unclear for most plant invaders.

Although plant-facilitated emissions have been demonstrated for one important invader (i.e., *P. australis*), there are few studies on other plant invaders or how plant-mediated emissions are influenced by different removal protocols or environmental factors. For instance, it was demonstrated that invasions by *Spartina alterniflora* directly (i.e., via soil) and indirectly (i.e., via plant-mediated mechanisms) promoted increases in CH_4 emissions [28]. Moreover, although the removal of invasive alien plants is often desirable [29], this may cause undesirable outcomes and secondary impacts on ecosystems [30]. The increased CH_4 emissions associated with vegetation removal by cutting [25] is one example of this. Nevertheless, the generality of this finding is still uncertain, as is how this may be impacted by other environmental factors such as waterlogging.

The aim of this study was therefore to evaluate whether *Gunnera tinctoria* Molina (Mirb.) directly or indirectly affects CO_2 and N_2O emissions and how this is influenced by waterlogging and/or leaf removal. *G. tinctoria* is an important N-fixing invasive alien plant species associated with significant negative impacts on ecosystems in Ireland and elsewhere [31]. Whilst we previously showed that invasions by *G. tinctoria* are associated with reductions in soil CO_2 emissions but had little impact on N_2O emissions [8], the contribution of the standing vegetation was not assessed. We hypothesized that flooding and the removal of plant parts would have contrasting effects on CO_2 and N_2O emissions. The removal of leaves would significantly reduce photosynthesis, whilst direct N_2O emissions from plants and soils would increase. Although the mechanism(s) associated with the production of N_2O by plant tissues is not known [32], the ability of *G. tinctoria* to fix atmospheric N [33–35], could also contribute to increased emissions [36]. Finally, we also hypothesized that waterlogging would reduce photosynthesis in intact plants, primarily through stomatal closure as well as resulting in plant-enhanced [32] and/or increased soil N_2O emissions [19].

2. Materials and Methods

2.1. Experimental Setup

To assess the impact of *G. tinctoria* on GHG fluxes, we collected 48 individual plants from invasive populations on Achill Island (53°51′ and 54°01′ N; and 9°55′ and 10°15′ W), Co. Mayo, west coast of Ireland. The plants were transplanted into 10 L plastic pots containing a mixture of 50% John Innes No. 2 compost (Westland Garden Health, Dungannon, UK) together with 50% of a multipurpose compost (pot plant substrate plus from the Bord na Móna® professional range) and a topsoil layer (*c.* 5 cm), originally from the island. Individuals were grown in a glasshouse at the University College Dublin Rosemount Environmental Research Station. The plants were divided into the following treatments: CON = control plants subjected to normal watering; WAT = plants subjected to flooding; CUT = plants that had the leaves cut from the petioles but were watered normally; CW = plants where leaves were removed and were also subjected to flooding by maintaining the water level above the rhizome.

Each 10 L pot, containing one plant, was placed into another larger plastic pot (29 cm in diameter) that contained a plastic bag. This enabled us to impose the flooding treatments and prevented gaseous exchange with the external environment during the GHG determinations with the photoacoustic gas analyser. The flooded plants were watered manually twice a day (morning and afternoon) maintaining a soil moisture content above 80%. Plants that were freely drained were manually irrigated once a day and maintained at a soil moisture content between 50–60%. We cut the leaves of the plants in the leaf removal treatments at the end of petioles (i.e., base of leaf) using pruning secateurs. Newly emerging leaves were clipped, as necessary.

2.2. Determination of Greenhouse Gas Fluxes

To determine CO_2 and N_2O fluxes, we enclosed whole *G. tinctoria* plants and soil (i.e., rhizome with roots, petioles, leaves and soil) inside two 66 L acrylic transparent cylindrical chambers (1 m high and 30 cm in diameter) with two tubes inserted into the top to connect with a photoacoustic gas analyser, model 1412, INNOVA-AirTech Instruments (LumaSense Technology A/S, Ballerup, Denmark). These are subsequently termed the mesocosm experiments. Each of the chambers contained a mini fan to circulate air. Soil CO_2 and N_2O fluxes were measured separately with the photoacoustic analyser using a 0.25 L polyvinyl chloride (PVC) cylindrical chamber fixed to a 10 cm PVC collar inserted into each plant pot to a depth of approximately 7.5 cm. We determined carbon dioxide and nitrous oxide fluxes before imposing the treatments, and then one day, one week, and one month (i.e., 0, 1, 7, and 30 days) after the application of the respective treatments, between July and August 2017.

To provide an airtight seal during the measurements on whole plants, the plastic bag was pulled out of the pot. Gas sampling with the photoacoustic gas analyser started when the 10 L pot, containing the individual plant and plastic bag, was enclosed using the 66 L acrylic chamber (see Supplementary Figure S1). All measurements were made within 20 min, at intervals of 0, 5, 10, 15, and 20 min after the system had been closed. For the separate measurements of soil fluxes using the small chamber, these were carried out within 5 min of inserting the chamber in the pot. Based on initial trials, using 5- and 20-min intervals, we avoided problems due to saturation of the gas concentration and ensured that the fluxes were linear for both chambers used.

2.3. CO_2 and N_2O Flux Calculations

We first calculated the GHG fluxes for the total mesocosm system (i.e., rhizome with roots, petioles, leaves, and soil). To estimate plant-mediated GHG fluxes (i.e., rhizome with roots, petioles, and leaves), we subtracted the soil fluxes from the total mesocosm system fluxes. The percentage contribution to the total GHG fluxes for both gases derived either from the soil or plant was calculated by dividing the total mesocosm GHG fluxes by the fluxes that came exclusively from the plants or soil. Fluxes were calculated following

a previous assessment on invasive populations of *G. tinctoria* [8] and were based on the surface area of the soil in the pot.

2.4. Environmental Variables

Data on soil moisture and temperature were collected using a WET-2 WET sensor (Delta-T Devices Ltd., Cambridge, UK) before the measurements of GHG fluxes and whenever individuals were sampled (i.e., 0, 1, 7, and 30 days). Photosynthetically active radiation (i.e., 400–700 nm) was measured with a SpectroSense 2 sensor (Skye Instruments Ltd., 2007, Llandrindod Wells, Powys, UK) at each measurement time. Soil moisture levels for waterlogged and normally watered plants averaged 81 ± 1.57% and 56 ± 0.27%, respectively. Soil temperature was similar amongst all treatments and/or sampling times with an average of 23 ± 0.21 °C throughout the whole experiment. Light levels varied between 700–996 μmol m^{-2} s^{-1}, with an average of 874 ± 7.61 μmol m^{-2} s^{-1}.

2.5. Plant Parameters

Data on leaf number, total leaf area, petiole area, and rhizome area were collected before each sampling time for each individual plant. The conversion of linear dimensions, estimated by measuring tape and/or caliper ruler, to the actual foliar area was determined from photographs analysed with Easy Leaf Area® software [37] following a previous assessment on invasive *G. tinctoria* populations [35]. We calculated leaf areas using the equation, Area = ((0.7276 × length × breadth) + 19.538; r^2 = 0.981). We estimated the area of petioles and rhizomes by considering them as cylinders and using the equation of cylinder surface area (A = 2π × radius × length + 2π × $radius^2$). The area of leaves and petioles were summed to estimate the total leaf area (m^2) and total petiole area (m^2) for each plant. The total petiole and rhizome areas were added to assess the contribution of whole plants to GHG fluxes.

2.6. Statistical Analysis

General linear mixed-model effects analysis was performed to check for differences on sampling times and their interaction with treatments regarding total, soil, and plant-mediated CO_2 and N_2O fluxes, with treatments (CON, WAT, CUT, and CW) as fixed factors and pots as random factors. The same approach was used to evaluate differences on soil moisture and temperature and plant parameters. Bonferroni's *post-hoc* correction was used to compare pair-wise differences and visual analysis of the residuals was carried out to ensure a normal distribution. Data on CO_2 fluxes for the whole mesocosm and plant-associated results were transformed on Log(x + 3) and data on N_2O fluxes and soil CO_2 fluxes were log transformed prior to analysis. Linear regression analyses were performed to verify the relationship between plant parameters and GHG fluxes. All analyses were performed with a significance level of $p = 0.05$, using SPSS Statistics v. 24 [38].

3. Results

3.1. CO_2 Fluxes

Considering the whole mesocosm measurements, there was an interaction between treatments and sampling times ($F_{(9,132)} = 11.06$; $p < 0.001$), but no differences were recorded across the treatments before these were applied ($p = 0.999$), with an average uptake of −1.36 ± 0.06 g CO_2 m^{-2} h^{-1} (Figure 1A). One day after leaf removal, significant carbon dioxide emissions were found in both the CUT (95% CI = 1.13, 2.28) and CW (95% CI = 1.08, 2.04) treatments, respectively, in comparison to CON (Figure 1A). The highest CO_2 emissions (1.02 ± 0.14 g CO_2 m^{-2} h^{-1}) were found in the CUT treatment after 30 days (95% CI = 0.77, 1.27; Figure 1A). For the WAT, there was a gradual reduction in CO_2 uptake over the course of the experiment, which was 21-fold smaller (−0.06 ± 0.23 g CO_2 m^{-2} h^{-1}; 95% CI = −0.31, 0.19) when compared to the start of the study (−1.27 ± 0.10 g CO_2 m^{-2} h^{-1}; 95% CI = −1.52, −1.02; Figure 1A). Similarly, there was a three-fold reduction in CO_2 uptake in CON (95% CI = −0.65, −0.15) in comparison to the beginning of the experiment

(95% CI = −1.67, −1.17). However, CON still had lower CO_2 emissions than CUT (−1.42, 95% CI = −1.90, −0.93) and CW (−0.81, 95% CI = −1.29, −0.33) at the end of the experiment. The pattern of CO_2 fluxes associated with *G. tinctoria* plants (Figure 1B) followed that described for the whole mesocosm measurements (Figure 1A). However, soil CO_2 emissions were significantly higher in CON at 1, 7, and 30 days ($F_{(9,132)}$ = 3.012; p = 0.003; Figure 1C).

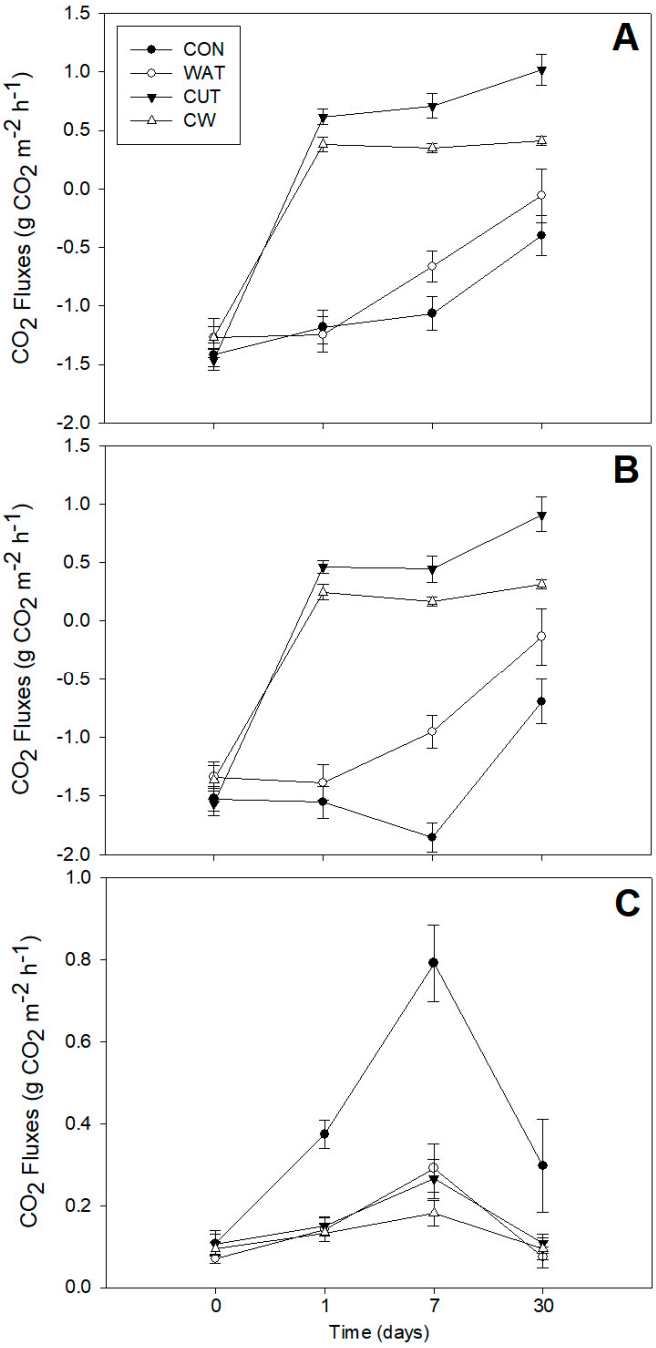

Figure 1. Whole mesocosm (**A**), plant-associated (**B**), and soil-associated (**C**) CO_2 fluxes at different sampling times (0, 1, 7, and 30 days), in experiments on *G. tinctoria* conducted at UCD's Rosemount Environmental Research Station glasshouse, Dublin, Ireland, July–August 2017 (n = 12; mean ± SE). Legend: CON = control plants subjected to normal watering; WAT = plants subjected to flooding; CUT = plants that had the leaves cut from the petioles but were watered normally; CW = plants where leaves were removed and were also subjected to rhizome flooding. Note: Negative numbers indicate absorption/uptake of CO_2, whereas positive numbers indicate emissions of CO_2.

Before the application of any treatments, CO_2 emissions originating exclusively from soil ranged from 5.5 to 8.8% across all treatments (Figure 2). During the experiment, there was a small increase in soil CO_2 emissions in all treatments, reaching a maximum after seven days, although the values at the end of the experiment were comparable to those at the beginning (Figure 2). After the application of the different treatments, most of the CO_2 fluxes were derived exclusively from *G. tinctoria* plants, with their contribution to the fluxes increasing with time, but varying amongst treatments. Whilst CO_2 uptake in CON was reduced throughout the experiment, all the other treatments resulted in significant emissions, although the patterns varied. Significant emissions were found one day after leaf removal, irrespective of waterlogging, in the CUT and CW treatments, whilst net emissions were only found after 30 days in WAT.

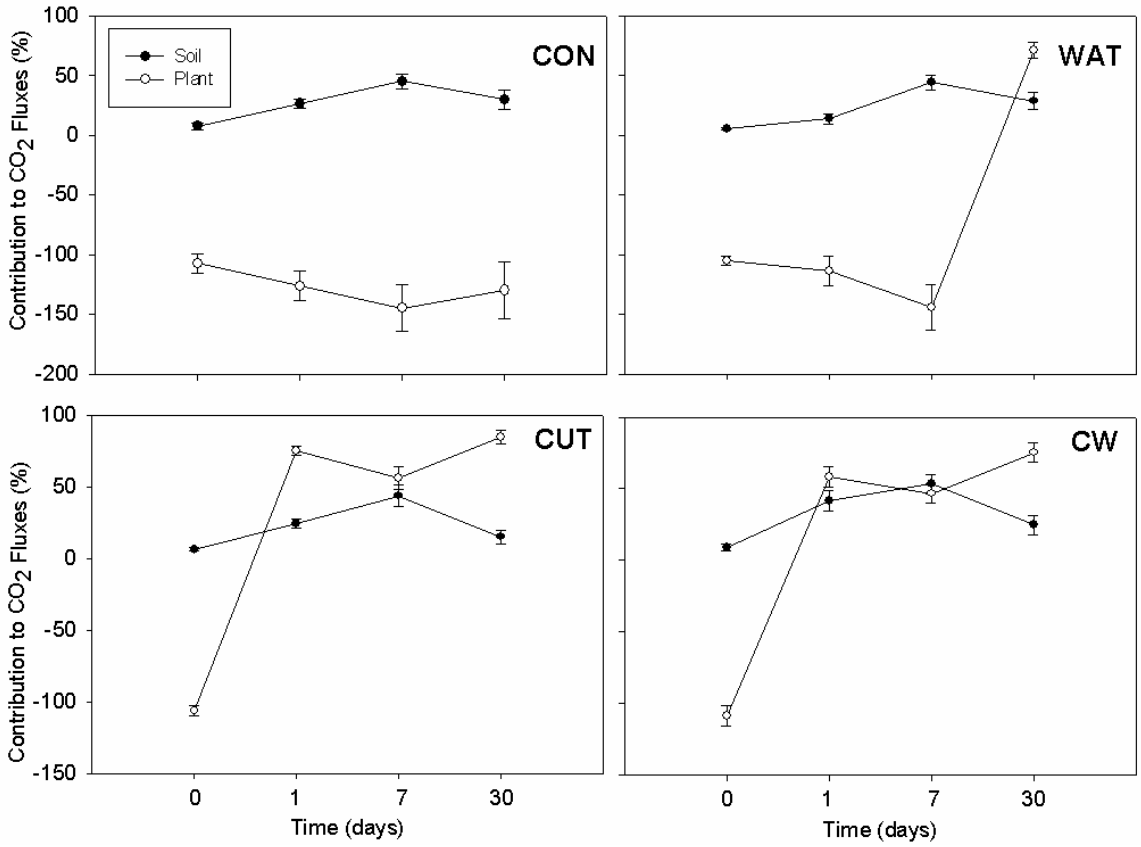

Figure 2. Percentage contribution of the soil or plant-related CO_2 fluxes to the total mesocosm CO_2 fluxes, in the different treatments at varying sampling times (0, 1, 7, and 30 days), in experiments conducted on *G. tinctoria* at UCD's Rosemount Environmental Research Station glasshouse, Dublin, Ireland, July–August 2017 (n = 12; mean ± SE). Legend: CON = control plants subjected to normal watering; WAT = plants subjected to flooding; CUT = plants that had the leaves cut from the petioles but were watered normally; CW = plants where leaves were removed and were also subjected to rhizome flooding. Note: negative numbers indicate absorption/uptake of CO_2, whereas positive numbers indicate emissions of CO_2.

3.2. N_2O Fluxes

Nitrous oxide fluxes varied significantly with time ($F_{(3,210)} = 21.03$; $p < 0.001$), but none of the treatments were significantly different from each other ($F_{(9,210)} = 1.85$; $p = 0.061$; Figure 3A) at any sampling time. Whilst N_2O emissions stayed in the same range for all sampling times in CON, with an average of 264.5 ± 1.18 μg N_2O m^{-2} h^{-1} (Figure 3A), WAT had its highest emissions at seven days (435.9 ± 89.73 μg N_2O m^{-2} h^{-1}; 95% CI = 330.2, 540.9; Figure 3A). Leaf removal treatments led to higher emissions, and CUT had its highest emissions one day after leaf removal (482.5 ± 143.55 μg N_2O m^{-2} h^{-1}; 95% CI = 380.4, 596.7) and CW at one week after the treatment was applied (489.8 ± 41.03 μg N_2O m^{-2} h^{-1};

95% CI = 384.4, 595.5; Figure 3A). Plant-mediated N_2O emissions followed the same pattern (Figure 3B) described for total emissions (Figure 3A). On the other hand, we found differences between the treatments for soil N_2O emissions ($F_{(9,132)} = 2.52$; $p = 0.011$; Figure 3C), and at seven days, CW showed higher soil N_2O emissions (95% CI = 7.19, 148.4) than CON.

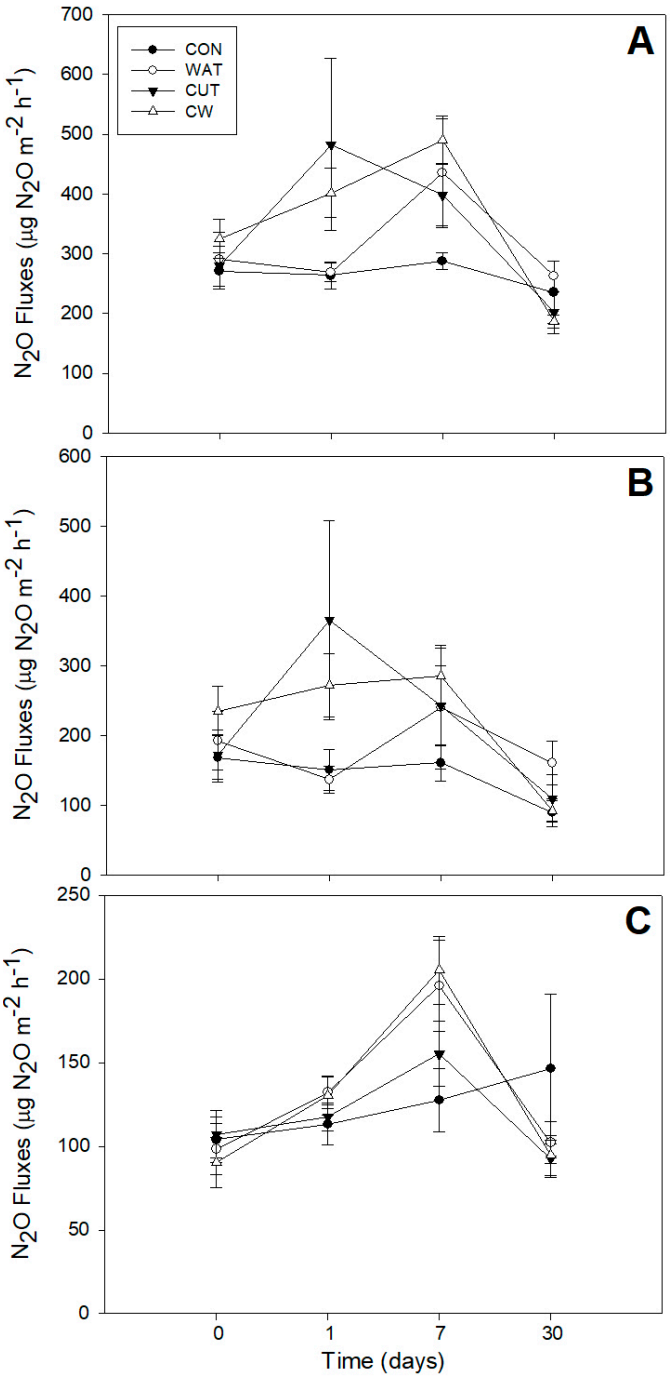

Figure 3. Whole mesocosm (**A**), plant-associated (**B**), and soil-associated (**C**) N_2O fluxes in the different treatments at varying sampling times (0, 1, 7, and 30 days), in experiments conducted on *G. tinctoria* at UCD's Rosemount Environmental Research Station glasshouse, Dublin, Ireland, July–August 2017 (n = 12, mean ± SE). Legend: CON = control plants subjected to normal watering; WAT = plants subjected to flooding; CUT = plants that had the leaves cut from the petioles but were watered normally; CW = plants where leaves were removed and were also subjected to rhizome flooding.

Before the treatments were applied, the majority of N_2O emissions were associated with *G. tinctoria* plants (average of 60% across all treatments; Figure 4). After the application of the treatments, we registered a steady increase in soil N_2O emissions with a concomitant reduction in plant-mediated N_2O emissions (Figure 4). In CON, CUT, and CW, there was an increase of *c.* 15% in soil N_2O emissions after 30 days in comparison to the beginning of the experiment (Figure 4). The WAT treatment was the exception, and after 30 days, most of the N_2O emissions were plant-mediated (55%; Figure 4).

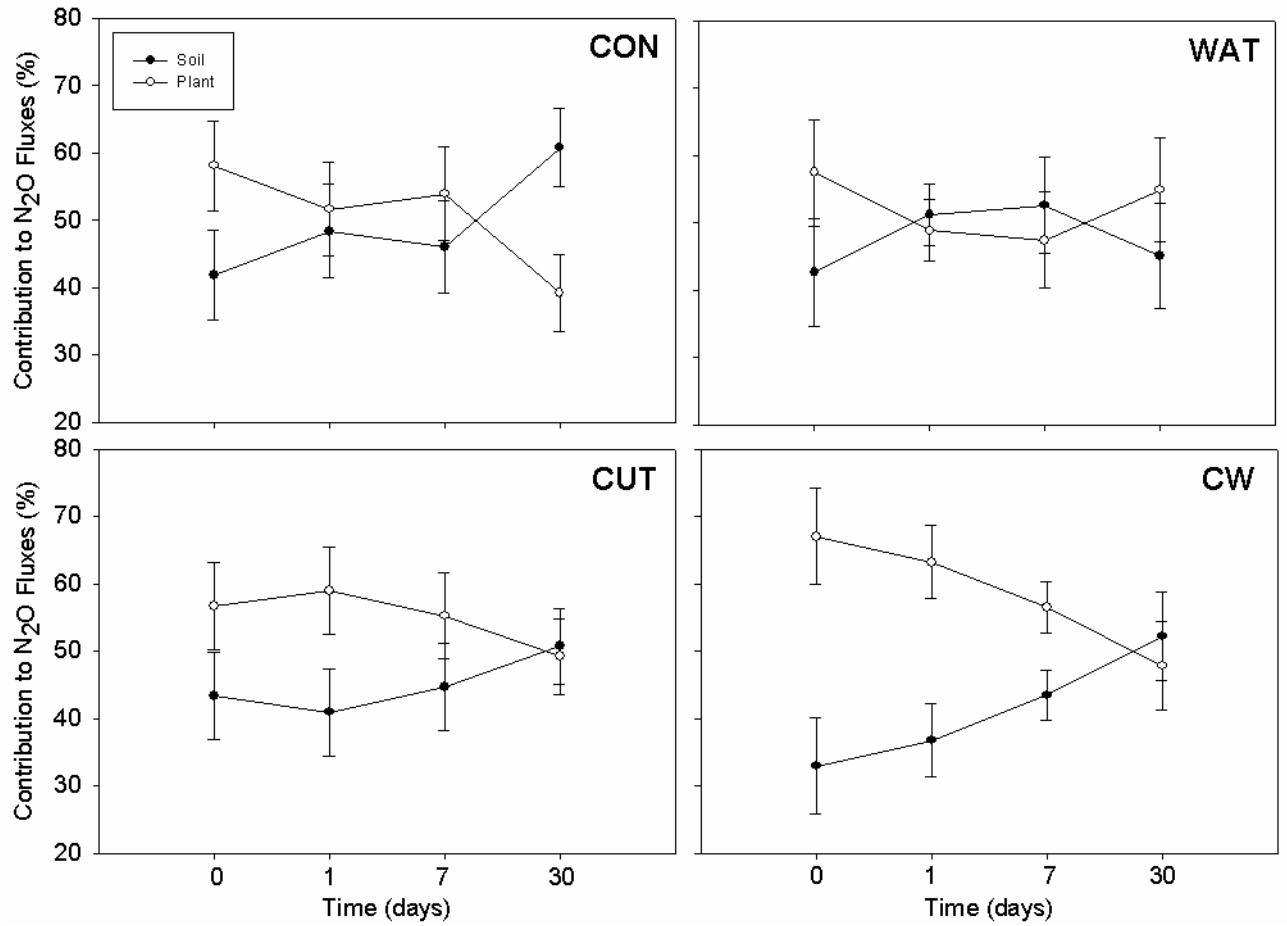

Figure 4. Percentage contribution of the soil or plant-related N_2O fluxes to the total mesocosm N_2O fluxes in the different treatments at varying sampling times (0, 1, 7, and 30 days), in experiments conducted on *G. tinctoria* at UCD's Rosemount Environmental Research Station glasshouse, Dublin, Ireland, July–August 2017 (n = 12; mean ± SE). Legend CON = control plants subjected to normal watering; WAT = plants subjected to flooding; CUT = plants that had the leaves cut from the petioles but were watered normally; CW = plants where leaves were removed and were also subjected to rhizome flooding.

3.3. Plant Data and Correlations with CO_2 and N_2O Fluxes

The total leaf area of intact plants varied with time ($F_{(9,132)} = 24.24$; $p < 0.001$), and at 30 days, CON showed a reduction of 35% (−0.13, 95% CI = −0.23, −0.04). In comparison, WAT lost 75% of its original leaf area (−0.29, 95% CI = −0.38, −0.19), and the *post-hoc* comparison showed that CON still had higher values (0.14, 95% CI = 0.19, 0.26) than the waterlogging treatment. No significant differences were found in the petiole area ($F_{(3,44)} = 1.24$; $p = 0.306$) and/or total rhizome area ($F_{(3,44)} = 1.04$; $p = 0.38$) between any of the treatments for any sampling time.

Both the CO_2 and N_2O fluxes for the total mesocosm measurements were significantly correlated with those associated with *G. tinctoria* plants across all treatments and sampling times, with r^2 values ranging from 0.84 to 0.98 (Table 1). Total petiole and total rhizome + petiole areas were positively correlated with total and plant-related N_2O

emissions in the WAT treatment (Table 2). We also found positive correlations between total petiole area and CO_2 and N_2O emissions for individual sampling times. The total rhizome + petiole area as well as rhizome area alone were positively correlated with total CO_2 fluxes in CUT (Table 2). Total petiole area was correlated with the total and with plant-related CO_2 emissions in CUT, and rhizome + petiole area was correlated with total CO_2 emissions in CW (Table 2).

Table 1. Correlations between total and plant-mediated CO_2 and N_2O fluxes across all treatments in experiments conducted on *G. tinctoria* at UCD's Rosemount Environmental Research Station glasshouse, Dublin, Ireland, July–August 2017. Legend: 0, 1, 7, and 30 refer to before, 1, 7, and 30 days after the application of treatments; *** indicates $p < 0.001$; # indicates the exclusion of one outlier from the analysis.

Time (Days)	CO_2 Fluxes	N_2O Fluxes	r^2	*p*
0	y = 1.001x − 0.094		0.965	***
		y = 0.96x − 88.27	0.850	***
1	y = 1.047x − 0.184		0.983	***
		y = 1.04x − 124.43	0.983	***
7	y = 1.17x − 0.354		0.917	***
		y = 0.904x − 132.02	0.851	***
30	y = 1.052x − 0.156		0.929	***
		y = 0.955x − 88.20 #	0.837	***

Table 2. Correlations between plant parameters with CO_2 and N_2O fluxes in experiments conducted on *G. tinctoria* at UCD's Rosemount Environmental Research Station glasshouse, Dublin, Ireland, July–August 2017. Legend: 0, 1, 7, and 30 refer to before, 1, 7, and 30 days after the application of treatments; WAT = plants subjected to flooding; CUT = plants that had the leaves cut from the petioles but were watered normally; CW = plants where leaves were removed and were also subjected to rhizome flooding. * $p < 0.05$; ** $p < 0.01$.

Time (Days)	Treatment	Variable	Total CO_2	Plant-Mediated CO_2	Total N_2O	Plant-Mediated N_2O	r^2	*p*
1	WAT	Petiole Area			y = 1090x + 187		0.35	*
						y = 1357x + 34.88	0.38	*
		Rhizome + Petiole Area			y = 981x + 164		0.36	*
						y = 1341x − 5.83	0.47	*
	CUT	Petiole Area	y = 6.08x + 0.148	y = 5.23x + 0.064			0.58	**
					y = 13,573x − 558		0.57	**
							0.59	**
						y = 13,376x − 660	0.57	**
		Rhizome Area	y = 20.14x − 0.069				0.50	**
		Rhizome + Petiole Area	y = 5.53x + 0.003	y = 4.52x − 0.035			0.66	**
					y = 10,940x − 727		0.59	**
							0.53	**
						y = 10,665x − 814	0.51	**
7	WAT	Rhizome Area				y = 18,914x − 350	0.35	*
	CUT	Petiole Area	y = 7.44x + 0.133	y = 7.55x − 0.141			0.41	*
							0.36	*
	CW	Rhizome + Petiole Area	y = 2.001x + 0.108				0.35	*

4. Discussion

4.1. CO_2 Fluxes

Our objectives with these experiments were to evaluate whether *G. tinctoria* directly or indirectly affects CO_2 and N_2O emissions. In addition, we wanted to examine how these could be influenced by waterlogging and/or leaf removal. Specifically, we proposed

that flooding and the removal of plant parts (i.e., leaf cutting) would have contrasting effects on CO_2 and N_2O emissions. We envisaged that leaf removal/waterlogging would significantly reduce photosynthetic CO_2 uptake, whilst N_2O emissions from plants and soils would increase.

The higher CO_2 emissions associated with leaf removal is an obvious effect linked to the elimination of photosynthesis, so that respiratory processes dominate. The lowest CO_2 fluxes (emissions) were associated with leaf removal when it was combined with waterlogging, which was also associated with a greater reduction in CO_2 uptake. This could possibly be associated with differences in respiratory activity with lower values for the rhizome under waterlogged and low oxygen conditions. Although soil CO_2 emissions were similar in the leaf removal and waterlogged treatments, surprisingly high values were found in the controls. Whilst the reason for this is not known, this could be related to the greater supply of labile C to the potting medium as a substrate to support soil respiration [39], where plant metabolism is not constrained by the removal of leaves and/or waterlogging. However, we did not measure soil respiration in the absence of roots (heterotrophic respiration), so it is unclear as to what extent the effect was due to an increased supply of labile C or whether the removal of leaves and/or waterlogging impacted directly on root (autotrophic) respiration. Nonetheless, the effect shows a clear increase followed by a decrease toward the end of the experimental period, perhaps indicating an effect associated with the loss of leaves (35%) in the control treatments. Leaf growth and expansion in *G. tinctoria* is particularly sensitive to soil and atmospheric water deficits [31] and the loss of leaves occurred during a 7-day period of exceptionally high temperatures (>35 degrees) and low humidity in the glasshouse.

The higher CO_2 fluxes registered in the waterlogging treatment after 30 days were similar to the results described for *Gunnera perpensa* [40], where more pronounced increases were associated with flooding. This reinforces the idea that *G. tinctoria* does not tolerate prolongated periods of waterlogging [31]. Consequently, waterlogging of invasive *G. tinctoria* populations could lead to enhanced GHG emissions, since more than 70% of CO_2 emissions were plant-derived after the exposure of plants to flooding for 30 days.

4.2. N_2O Fluxes

Intact plants of *G. tinctoria* were a source of N_2O emissions and this increased with leaf removal and waterlogging. Although there are now several examples of direct plant-associated N_2O emissions, the mechanism(s) is still unknown [32]. Earlier studies emphasized the role of plants as passive conduits for the movement of gases including N_2O and CH_4 from the soil to the atmosphere [11–15], although this may have been confounded by direct emissions from plant tissues [19]. In our studies, the involvement of passive diffusion cannot be ruled out and *G. tinctoria* does have an extensive aerenchyma system [31] that could facilitate gaseous transport. However, the increase in emissions after removing leaves might suggest that there is a significant resistance to gas exchange in intact plants that limits gas movement.

Conversely, there is increasing evidence that different plant parts [19] can produce N_2O directly through processes that may be linked to mitochondrial activity ([32], and references therein). Interestingly, the *Nostoc* species may be associated with increased N_2O production [41] so that symbiotic *N. punctiforme*, present in the rhizome tissue of *G. tinctoria*, may be a source of this gas [33,34]. Given that the symbiotic *Nostoc* are the sites of N fixation, this may indicate that reactions associated with nitrogen fixation in general can be a source of N_2O [36]. If a direct mechanism is involved, the higher values obtained after removing leaves would suggest that this is due to the release of gas generated internally within plant tissues. Based on the strong correlations between N_2O emissions and plant/organ size, the mechanism involved could be attributed to either direct production by plant tissues and/or indirect gaseous root-to-shoot movement. Importantly, the effect was size-dependent with larger emissions associated with increased size, which may be of particular relevance for management decisions on the control or eradication of plant invaders. Clearly, the

removal of young plants during the early establishment phase would reduce the impact that *G. tinctoria* invasions would have on atmospheric N_2O emissions.

Water availability also influenced soil N_2O emissions, with higher emissions in the waterlogged treatments. Recent evidence suggests that short-term flooding can increase soil as well as plant-related N_2O emissions [19]. Increased denitrification, as a response to root exudates [42], after damage to leaves, can also lead to enhanced N_2O emissions [43]. High N_2O pulses after wetting soils invaded by *Bromus tectorum* have also been found [44] as well as elevated N_2O emissions after rapid flooding [22], although this may also be dependent on nitrate availability [19]. The creation of waterlogging-related low oxygen conditions [45,46] might also have stimulated an increase in emissions if denitrification reactions were the major source of N_2O [10]. Additionally, the limited ability of *G. tinctoria* to utilise soil nitrate [47] may have resulted in more nitrates being available for conversion to N_2O.

5. Conclusions

The wider implications of our results are that an assessment of the totality of effects of *G. tinctoria* invasions on GHG emissions would need to account for vegetation-mediated nitrous oxide emissions. The fact that other species associated with wetland environments can also exhibit high CH_4 emissions [19] indicates that this GHG also needs to be assessed. Our previous in situ study [8] showing that soil N_2O emissions were largely unchanged would therefore have underestimated the full impact of *G. tinctoria* invasions on GHG budgets. Considering only the control plants and the N_2O emissions for the whole mesocosm during the 30 days of this experiment, *G. tinctoria* emitted, on average, 264 $\mu g\ N_2O\ m^{-2}\ h^{-1} \pm 16.29$. This would be roughly equivalent to 23.17 $kg\ N_2O\ m^{-2}\ y^{-1} \pm 1.43$. In our in situ experiments on soil GHG emissions [8], we found that areas invaded by *G. tinctoria* had emissions of 4.50 $kg\ N_2O\ m^{-2}\ y^{-1} \pm 3.47$, a value that was five-fold smaller than those reported in the current study. Although there may be difficulties in directly extrapolating the current experimental results on single plants to populations growing in the field, this does suggest that there could have been significant plant-related N_2O emissions that were unaccounted for. High plant-associated N_2O emissions could also have contributed to the absence of any significant increase in soil N concentration associated with invasive *G. tinctoria* populations [8,48]. To what extent high plant-related N_2O emissions is a common feature of other invasions is largely unknown, although one significant invader, *P. australis*, has been shown to facilitate GHG emissions by acting as a low resistance pathway for gaseous diffusion [11,14]. Consequently, any assessment of the effects of plant invasions on ecosystem GHG budgets would need to account for both soil and plant-related N_2O emissions.

There is increasing evidence that many plants may be able to directly facilitate GHG emissions, particularly under waterlogged or flooded conditions [19], although the mechanisms of such processes are unknown [32]. It is also unclear as to what extent plant invasions result in an alteration in ecosystem GHG budgets [8], and the extent to which plants in general, whether they are native or introduced, have some capacity to facilitate GHG emissions. Based on our studies, the removal of above-ground plant material could not only enhance vegetation-related N_2O emissions, but would, due to associated increases in net CO_2 emissions, exacerbate the effects that management interventions have on ecosystem GHG budgets.

Finally, although the extent to which the removal of above-ground vegetation impacts on both the CO_2 and N_2O budgets in situ is unknown, this is likely to depend on the standing plant biomass, and therefore differs between the early and later stages of the invasion process. The early removal of young plants would have less of an impact than the removal of older, more established plants. The impact on ecosystem GHG budgets could also depend on the plant parts removed. In our study, we only removed leaves, leaving the remaining petioles (equivalent to stems for this plant) intact, which may have prevented the initiation of new leaves during this experiment and a faster recovery of CO_2 uptake.

Similarly, it is unclear as to what extent the removal of all or some of the above-ground plant parts might have on N_2O emissions under natural conditions. This argues for more detailed assessments of the effects of above-ground removal techniques on ecosystem GHG emissions for this and other invasive plant species.

Supplementary Materials: The following is available online at https://www.mdpi.com/article/10.3390/d13090427/s1, Figure S1: Greenhouse gas emission sampling with a photoacoustic gas analyser at UCD's Rosemount Environmental Research Station glasshouse on July 2017, in CON (control) and CW (leaf removal + waterlogging). Photo: MC Mantoani.

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