



Article

Impact of Landfill Gas Exposure on Vegetation in Engineered Landfill Biocover Systems Implemented to Minimize Fugitive Methane Emissions from Landfills

Dinu S. Attalage ¹, J. Patrick A. Hettiaratchi ^{2,*}, Angus Chu ², Dinesh Pokhrel ² and Poornima A. Jayasinghe ²

¹ Department of Geoscience, University of Calgary, 2500 University Drive, NW, Calgary, AB T2N 1N4, Canada

² Department of Civil Engineering, Center for Environmental Engineering Research and Education (CEERE), University of Calgary, 2500 University Drive, NW, Calgary, AB T2N 1N4, Canada

* Correspondence: jhettiar@ucalgary.ca

Abstract: Engineered landfill biocovers (LBCs) minimize the escape of methane into the atmosphere through biological oxidation. Vegetation plays a crucial role in LBCs and can suffer from hypoxia caused by the displacement of root-zone oxygen due to landfill gas and competition for oxygen with methanotrophic bacteria. To investigate the impact of methane gas on vegetation growth, we conducted an outdoor experiment using eight vegetated flow-through columns filled with a 45 cm mixture of 70% topsoil and 30% compost, planted with three types of vegetation: native grass blend, Japanese millet, and alfalfa. The experiment included three control columns and five columns exposed to methane, as loading rates gradually increased from 75 to 845 gCH₄/m²/d over a period of 65 days. At the highest flux, we observed a reduction of 51%, 31%, and 19% in plant height, and 35%, 25%, and 17% in root length in native grass, Japanese millet, and alfalfa, respectively. The column gas profiles indicated that oxygen concentrations were below the levels required for healthy plant growth, which explains the stunted growth observed in the plants used in this experiment. Overall, the experimental results demonstrate that methane gas has a significant impact on the growth of vegetation used in LBCs.

Keywords: methane oxidation; landfill gas; plant stress; fugitive methane emissions; landfill biocover systems



Citation: Attalage, D.S.; Hettiaratchi, J.P.A.; Chu, A.; Pokhrel, D.; Jayasinghe, P.A. Impact of Landfill Gas Exposure on Vegetation in Engineered Landfill Biocover Systems Implemented to Minimize Fugitive Methane Emissions from Landfills. *Int. J. Environ. Res. Public Health* **2023**, *20*, 4448. <https://doi.org/10.3390/ijerph20054448>

Academic Editors: Jay N. Meegoda and Daniel Jay Watts

Received: 28 January 2023

Revised: 22 February 2023

Accepted: 27 February 2023

Published: 2 March 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Despite the practices of reducing, recycling, and reusing, considerable amounts of municipal solid waste (MSW) end up in sanitary landfills. MSW in sanitary landfills undergoes biochemical reactions and produces landfill gas (LFG), primarily comprised of the greenhouse gasses, methane (CH₄) and carbon dioxide (CO₂) [1–3]. Engineered landfill biosystems, such as landfill biocovers (LBCs), bio-windows, and methane biofilters populated with methanotrophic bacteria, or methanotrophs, with the capability of oxidizing CH₄ can potentially be used to reduce the fugitive methane emissions to the atmosphere [4,5]. Vegetation is incorporated in LBC design for erosion control, improvement of the aesthetic value, and to encourage evapotranspiration [6,7]. Furthermore, the presence of vegetation in LBCs alters the water balance and microbial community dynamics [8,9]. The condition of the vegetation on LBCs impacts the growth and activity of methanotrophs, and the vegetation type and coverage become determining factors in biological CH₄ oxidation [10,11]. Therefore, maintenance of a healthy growth of vegetation on LBCs is vital for efficient biological CH₄ oxidation. However, because of the potential hostile environment presented by LBCs, maintaining healthy vegetation is a serious challenge.

Several researchers have reported that LFG can severely inhibit plant growth on landfill surfaces [12,13]. In a study involving grass, vines, and herbs, a negative correlation between the vegetation cover on landfills and LFG emissions was observed [14]. Stunted

growth of corn (*Zea mays* Strurtev.) and sweet potato (*Ipomea batata* L.) was observed when the root zones of the plants were exposed to LFG [15]. Tao et al. [6] observed that species diversity of plants grown on restored landfill surfaces decreased over time, indicating the effect of LFG on vegetation growth is plant species specific. Other researchers have aimed at understanding the causes and mechanisms of plant distress encountered at landfills [6,15–20]. Toxicity of LFG, depleted oxygen (O₂), soil CO₂, thin layer of cover soil, nutrient deficiencies, low water holding capacity of cover material, high soil temperatures, high soil compaction, and the use of sensitive plants have been identified as key reasons for limited vegetation growth at landfill sites [21]. Early research has shown that an O₂ level above 10% in the soil-gas phase of the root zone is needed for the growth of most plants, and plant growth can be impacted at levels below 10–15% [19,22]. The escape of significant amounts of LFG across the landfill surface has the potential to displace O₂ in the root zone, thereby lowering the O₂ levels below the threshold levels [13,18]. However, Gilman et al. [16] found that certain plant species were able to withstand low O₂ tension in the soil and tolerated landfill conditions better than others. In addition to causing hypoxia that retards the growth of vegetation, the components of LFG themselves could also impact vegetation growth [15]. Past researchers have noted that although CH₄ is not phytotoxic, elevated levels of CO₂ can cause damage to roots even in the presence of adequate amounts of O₂ in the root zone [15,20].

Most past studies have focused on gaining an understanding of plant growth on impacted soil surfaces, including landfill final covers, and they have identified two factors that cause the stunted growth of plants: the lack of O₂ and the presence of high levels of CO₂ in the root zone. A key aspect that has not been studied in detail is the contribution of soil methanotrophy to plant stress. Biological oxidation of CH₄ in LFG by methanotrophs consumes O₂ in the root zone and also produces CO₂ [23], thereby potentially aggravating the problem of stunted plant growth. However, methanotrophic activity in the root zone of LBC is not evenly distributed and varies with depth [10]. Therefore, root zone hypoxia caused by methanotrophic activity may also show uneven distribution. Although existing studies have discussed the importance of vegetation on LFG emission reductions, the interaction between plants and methanotrophs in LBCs is still poorly understood [10]. Therefore, the key objective of the current research is to fill these knowledge gaps and provide critical information on plant behavior in the presence of LFG, for professionals involved in the implementation of engineered biosystems, such as LBCs, for mitigating landfill CH₄ emissions.

2. Materials and Methods

2.1. Study Location

The vegetated flow-through biofilter columns were set up outdoors at the Okotoks Eco-center research facility located in Okotoks, Alberta, Canada (50°42′58.78″ N and 113°57′0.94″ W). The facility is located in the prairie region of Canada, 1053 m above mean sea level and receives an annual average precipitation of 437 mm [24]. The climate is semi-arid and large temperature variations occur during summer and winter seasons, with mean minimum and maximum air temperatures ranging between −20.5 °C and 25.5 °C.

2.2. Flow-Through Vegetated Biofilter Columns

Eight high-density polyethylene (HDPE) flow-through biofilter columns of diameter 35 cm and height 50 cm were used for this study (see Figure 1). Each column was filled with a 5 cm layer of gravel at the bottom to ensure uniform gas distribution, followed by a 45 cm biofilter medium comprised of soil/compost mixture. To prevent potential clogging of the gravel layer, a non-woven geotextile layer was placed between the gravel and the biofilter medium. The biofilter medium was composed of 70% (v/v) topsoil sourced from a pile of topsoil (sandy lean clay) intended for use in a future landfill biocover at the Leduc regional landfill located in central Alberta, Canada, and 30% (v/v) residual compost obtained from the Edmonton waste management center (Edmonton, AB, Canada). The

physical and chemical characteristics of the compost and soil are shown in Table 1. The thickness of the biofilter medium was chosen to be 45 cm based on previous research, which demonstrated that the majority of methane oxidation takes place within a narrow zone of approximately 20 cm, located about 10–15 cm below the top surface of the medium.

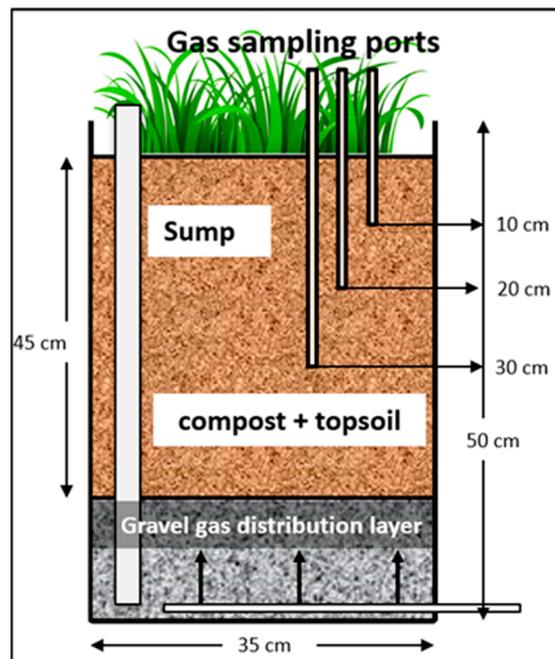


Figure 1. Schematic diagram of the constructed columns.

Table 1. Characteristics of column construction materials.

Parameters	Units	Topsoil	Residual Compost
Moisture content	%	22.74	28.77
Organic matter	%	5.89	49.53
Dry bulk density	(g/cm ³)	1.21	0.95
Field Capacity	%	45.06	164.94
C/N	ratio	15.30	10.57

Three types of plants were chosen in this study: native grass (a blend of 25% Northern wheat grass—*Elymus lanceolatus*, 35% Awnead wheat grass [*Elymus trachycaulus*], 10% June grass [*Koeleria macrantha*], 25% Tufted hair grass [*Deschampsia caespitose*], and 5% Rough fescue [*Festuca hallii*]; Japanese millet (*Echinochloa esculenta*); and alfalfa (*Medicago sativa*). Each vegetated column was irrigated at a rate of 1 L/week after establishing the plants. The native grass blend is the most common type of vegetation used in LBCs; Japanese millet and alfalfa were selected in this study because they are cash crops and there is some interest among landfill operators to vegetate closed landfills with cash crops to ensure landfill space is used for beneficial purposes. Furthermore, with different root systems and potential for millet species plants to tolerate hypoxia because of the formation of aerenchyma, there is the possibility that each of the three selected species will produce different types of responses during exposure to CH₄ gas.

2.3. Exposure of Vegetated Columns to Methane Gas

Five test columns and three control columns were used in this study. Three columns of native grass (triplicates), one column of Japanese millet, and one column of alfalfa were exposed to CH₄ gas, while the remaining three columns were used as control columns with no gas exposure for each vegetation type, as shown in Figure 2. Details of all the eight columns are given in Table 2.

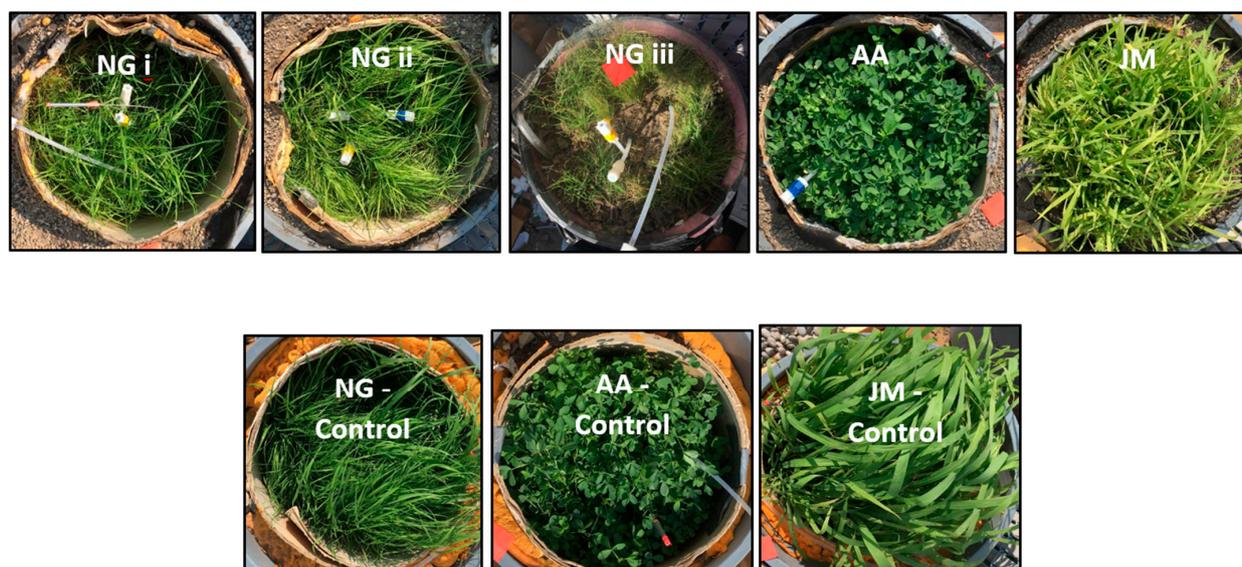


Figure 2. Experimental columns with different types of vegetative covers.

Table 2. Vegetation type and gas exposure details of columns.

Column ID	Type of Vegetation	Natural Gas Exposure
NG-i	Native grass	+
NG-ii	Native grass	+
NG-iii	Native grass	+
AA	Alfalfa	+
JM	Japanese Millet	+
NG-control	Native grass	—
AA-control	Alfalfa	—
JM-control	Japanese Millet	—

(+) sign indicates exposure to methane gas and (—) indicates control groups.

Over a 65-day period, natural gas containing 92% CH₄ was introduced from the bottom of the gas-exposure columns at a pressure of 5 psi. Over this time period, the gas loading rates were gradually increased in six steps, ranging from 75.1 to 845.2 gCH₄/m²/d. The loading rates were 75.1 gCH₄/m²/d for the first 7 days, 173.1 gCH₄/m²/d from day 7 to 27, 285.1 gCH₄/m²/d from day 27 to 35, 437.9 gCH₄/m²/d from day 35 to 41, 635.1 gCH₄/m²/d from day 41 to 62, and 845.2 gCH₄/m²/d from day 62 to 65. The ambient temperature varied from +9 °C to +26 °C during the experiment.

2.4. Determination of Plant Growth Characteristics

Plant growth characteristics, such as plant height and root length, were measured on day 35, day 50, and day 65 (at the end of the experiment). Plant height was measured from soil level to the terminal bud of a representative sample of plants and then averaged [25]. Similarly, root length was measured by collecting a representative sample of plants with minimum damage to the root systems and measuring the length from soil level to the tip of the root. At the conclusion of the column experiments on day 65, above-ground and below-ground dry biomass were measured by selecting sample plants from three areas (4 cm × 4 cm) of each column tested. To ensure the sample plants were representative of the population, the selection of these samples was performed in such a way as to include short, medium, and tall plants in each sample. The root systems were washed, cleaned, and separated from the rest of the plants. Both above-ground and below-ground plant tissue matter were separately dried in an oven at 60 °C for a minimum of 72 h and then weighed to calculate the plant above-ground and below-ground dry biomass density in g/cm² [26].

The chlorophyll contents of the leaves of plants were determined on day 65 by treating 0.5 g of fresh leaf samples in an 80% acetone solution [27]. The optical densities of the total chlorophyll (α and β) content were determined at specific wavelengths of 645 and 663 nm using a UV-VIS spectrophotometer (Model: UV-2600, Shimadzu Corporation, Kyoto, Japan). The total chlorophyll content was calculated using the formula in [28],

$$\text{Chlorophyll } a = \frac{12.3 D_{663} - 0.86 D_{645}}{d \times 1000 \times W} \times V \quad (1)$$

$$\text{Chlorophyll } b = \frac{19.3 D_{645} - 3.6 D_{663}}{d \times 1000 \times W} \times V \quad (2)$$

$$\text{Total Chlorophyll content} = \text{Chlorophyll } a + \text{Chlorophyll } b \quad (3)$$

where, V is the volume (mL) of acetone used for chlorophyll extraction, d is the light path length (cm), D_{663} is the absorbance at 663 nm, D_{645} is the absorbance at 645 nm, and W is the weight of the leaf used and the total chlorophyll content (a and b) in 1 g of sample leaf. To evaluate the significant differences in plant characteristics, such as height and root length at different growth stages between gas-exposed and control groups within each vegetation type, a two-way ANOVA was conducted. Additionally, t -tests were used to assess the significant differences between the gas-exposed and control groups in terms of vegetation characteristics, including above-ground and below-ground dry biomass, as well as chlorophyll content. The statistical analyses were performed using SigmaPlot 14.0 software from Systat Software Inc., and statistical significance was indicated where $p < 0.05$.

2.5. Determination of Gas Concentration Profiles in Columns

The gas concentration profiles of columns were measured on day 7, day 41, and day 65 by taking 5 mL samples from sampling ports located at 10, 20, and 30 cm depths using a luer-lock syringe with a two-way stainless-steel stop cock attached to a non-coring needle and then analyzing the samples for CH_4 , CO_2 , O_2 , and N_2 using an SRI 8610C (SRI Instruments, Torrance, CA, USA) equipped with a thermal conductivity detector as described by La et al. [5].

2.6. Methane Oxidation Assessment

The surface CH_4 emission fluxes of the test columns were measured using the static closed flux chamber method [29] to determine the rate and the efficiency of methane oxidation. The difference between the CH_4 influx and the surface CH_4 emission flux was used to calculate the rate of CH_4 oxidation. The CH_4 oxidation efficiency was calculated using the following equation as described by Powelson et al. [30],

$$\text{CH}_4 \text{ oxidation efficiency} = \frac{(\text{Flux}_{\text{loaded}} - \text{Flux}_{\text{emitted}})}{\text{Flux}_{\text{loaded}}} (100) \quad (4)$$

where, $\text{Flux}_{\text{loaded}}$ and $\text{Flux}_{\text{emitted}}$ are the CH_4 inlet and surface emission fluxes in $\text{gCH}_4/\text{m}^2/\text{d}$, respectively.

After the completion of column experiments on day 65, undisturbed core soil samples were collected at depths between 15 to 25 cm from each column using a soil probe to perform batch oxidation kinetic experiments. Samples weighing 10–20 g were incubated in 250-mL airtight amber serum bottles with a headspace CH_4 concentration of 10% [31]. The CH_4 concentrations were then measured using gas chromatography at several time intervals until zero. The oxidation rates were then determined by plotting the change in headspace CH_4 concentrations vs. time. The value of V_{max} [32] was obtained by using CH_4 oxidation rates to produce substrate saturation curves as a function of initial headspace CH_4 concentration. The data were then linearized with Eadie-Hofstee plots [31].

3. Results

In this section, first, the vegetation impacts during exposure of vegetated columns to CH₄ gas are presented. Thereafter, the gas concentration profiles of the columns and CH₄ oxidation results are presented in order to provide possible reasons for the vegetation impacts. The detailed discussion and interpretations of results, as well as the practical implications, are included in the next section.

3.1. Vegetation Growth Parameters with and without Gas Exposure

The growth parameters of the three plants; plant height, root length, and above-ground and below-ground plant dry biomass densities, with and without gas exposure, are shown in Figure 3a–d, respectively.

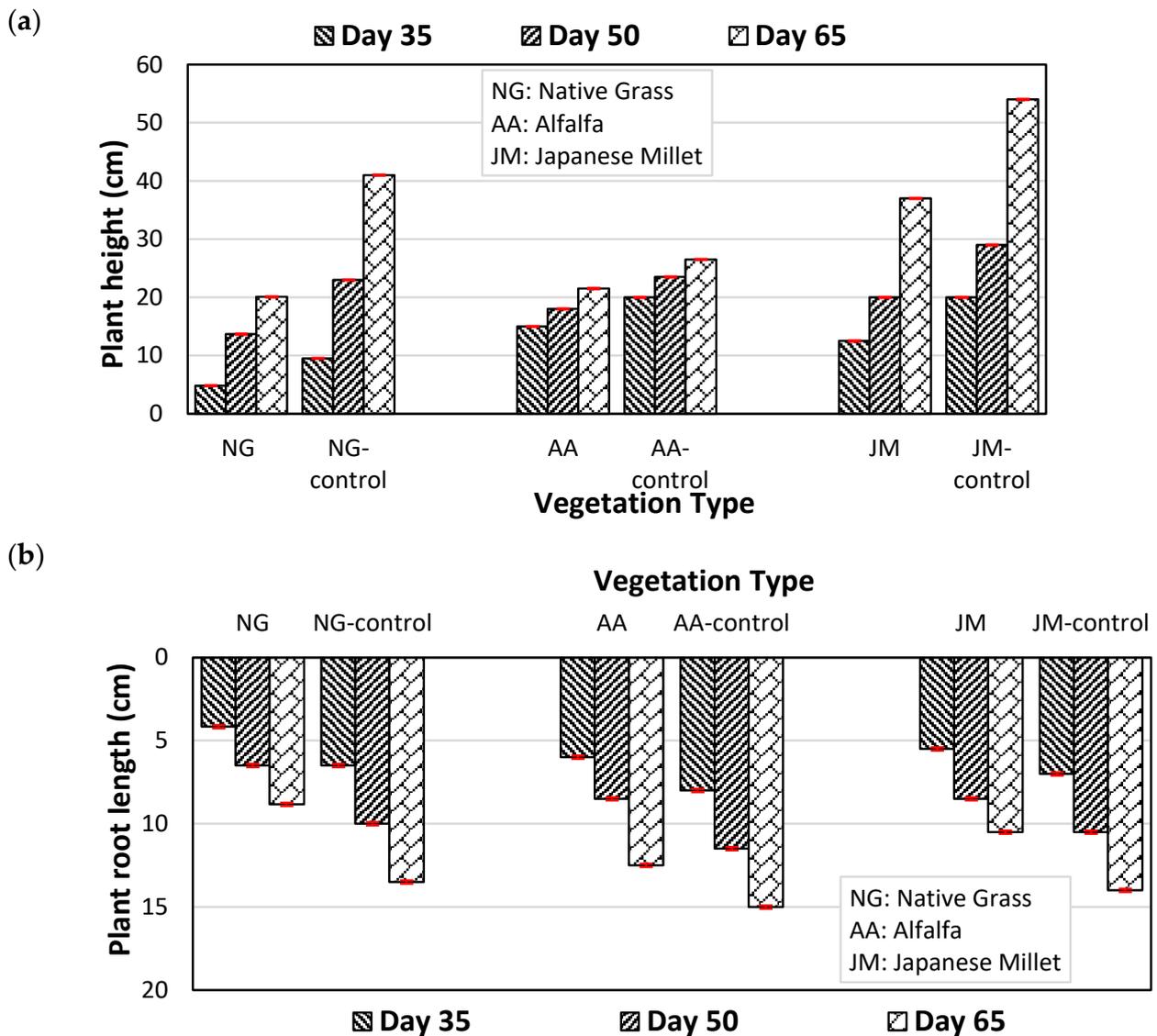


Figure 3. Cont.

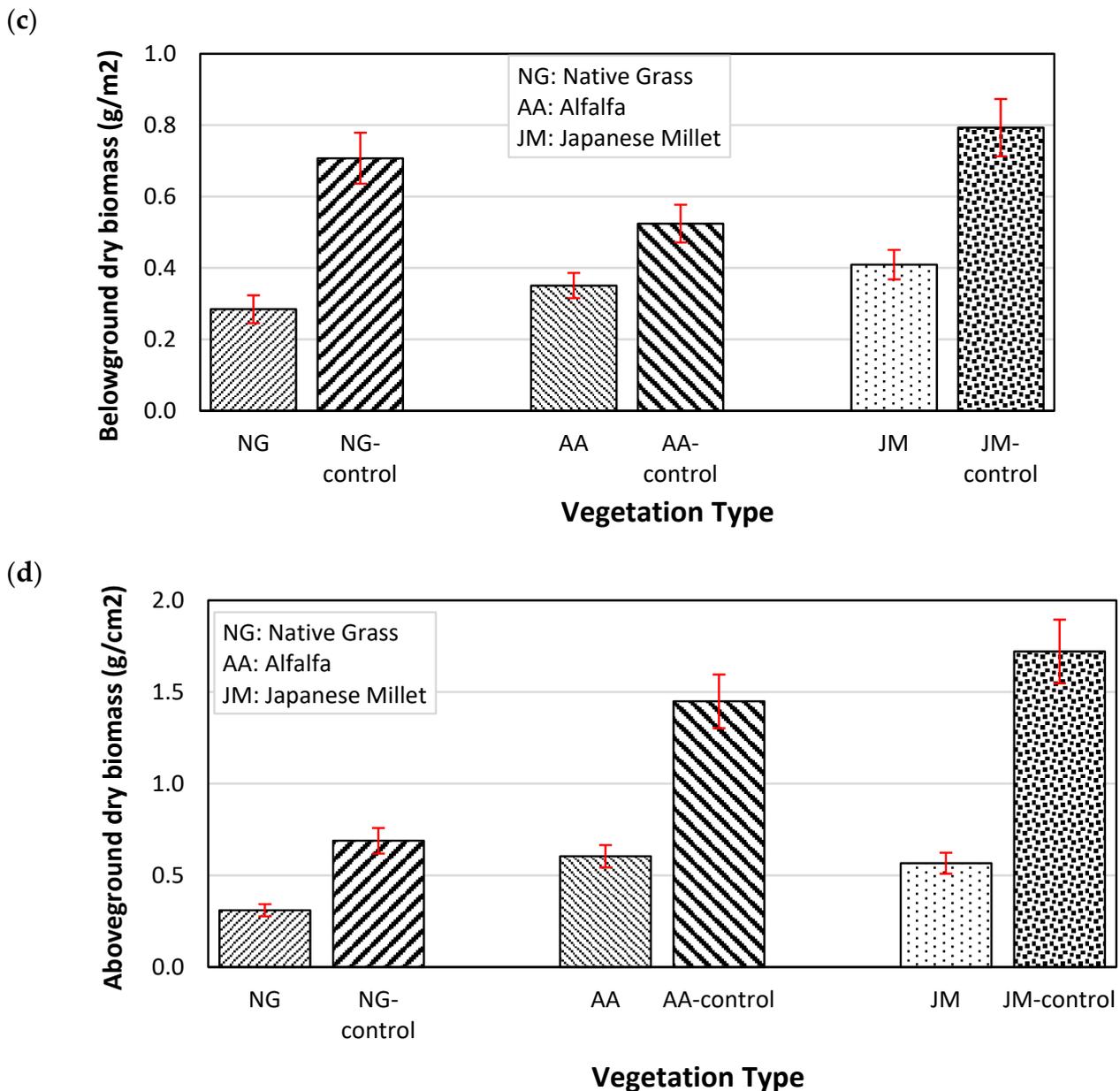


Figure 3. Comparison of vegetation characteristics under natural gas exposure and control (with no exposure); (a) Plant heights (b) Root lengths (c) Below-ground dry biomass densities (d) Above-ground dry biomass densities (NG: native grass, AA: alfalfa and JM: Japanese millet).

Figure 3a, representing the impact of CH₄ gas exposure on plant height, shows that the highest plant height reductions were observed in native grass, with average reductions for the three replicates of 49.2%, 40.5%, and 61.0% in comparison with control columns, on day 35, day 50, and day 65, respectively. The lowest corresponding reductions of 25.0%, 23.4%, and 18.8% were observed for alfalfa, whereas the reductions for Japanese millet were 37.5%, 31.0%, and 31.5%. Similarly, as shown in Figure 3b, root length decreases of 35.9%, 35.0%, and 34.6% were observed for native grass after day 35, day 50, and day 65, respectively. Corresponding reductions were 25.0%, 26.1%, and 16.7% for alfalfa, and 21.4%, 19.0%, and 25.0% for Japanese millet. The results of the two-way ANOVA revealed statistically significant differences in plant height between the gas-exposed and control columns in both the native grass and alfalfa groups, with p -values of $p = 0.049$ for NG-gas vs. NG-control and $p < 0.001$ for AA-gas vs. AA-control. Similarly, the analyses showed statistically significant differences in root length between the gas-exposed and control

columns in both vegetation types, with p -values of $p < 0.001$ for NG-gas vs. NG-control and $p = 0.013$ for AA-gas vs. AA-control.

As shown in Figure 3c,d, the below-ground dry biomass densities after 65 days of native grass, alfalfa, and Japanese millet were 0.28 (average of three replicates), 0.35, and 0.41 g/cm², respectively, while the control groups had densities of 0.71, 0.52, and 0.79 g/cm² for native grass, alfalfa, and Japanese millet, respectively. The above-ground dry biomass densities of gas-exposed native grass, alfalfa, and Japanese millet at the end of the experimental period (i.e., over the full 65 days) were 0.31 (average of three replicates), 0.60, and 0.57 g/cm², respectively, while the dry biomass densities for the control groups were much higher, with values of 0.69, 1.45, and 1.72 g/cm² for native grass, alfalfa, and Japanese millet, respectively. Consistent with previous findings, native grass exhibited the largest impact, while alfalfa had the least impact on both above- and below-ground dry biomass densities. The results of the student T -tests conducted on above-ground and below-ground dry biomass and chlorophyll content indicated no statistically significant differences between the growth of natural grass, alfalfa and Japanese millet in the gas-exposed and control columns.

The chlorophyll content of the three types of vegetation with gas exposure and control columns (i.e., with no gas exposure) is shown in Figure 4. The amount of total chlorophyll decreased in each type of vegetation exposed to CH₄ gas when compared with the control columns, and native grass blend showed the least impact. Total chlorophyll content reductions of 24.4%, 29.8%, and 40.2% were observed for native grass (average of three replicates), alfalfa, and Japanese millet, respectively. Past research studies have shown that plants under stress develop chlorosis, or a yellowing of leaves, because of low O₂ concentrations in the root zone [33,34]. Low O₂ concentrations in the root zone inhibit chlorophyll biosynthesis, causing a decline in chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content [34]. This reduction in chlorophyll content under O₂-deficit conditions can affect the rate of photosynthesis in plants, and therefore, plant growth as well [35].

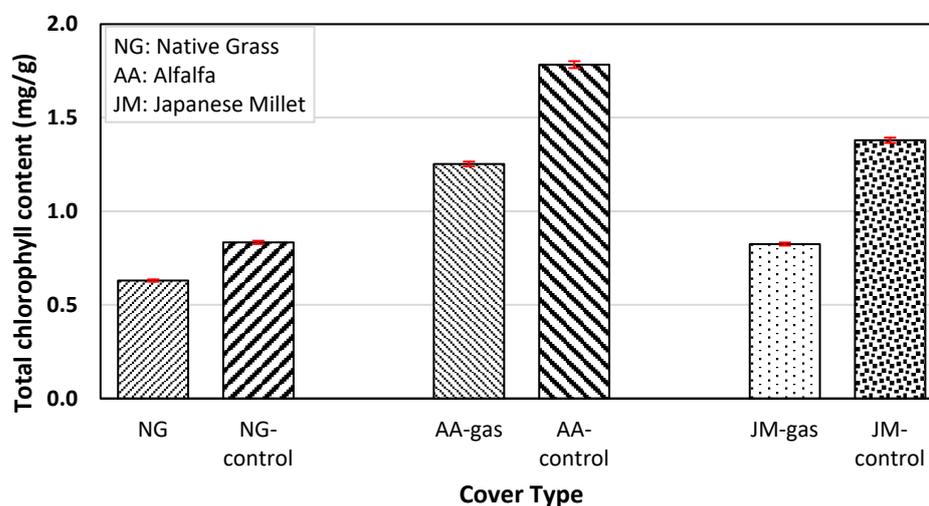


Figure 4. Chlorophyll content of different types of vegetation under two conditions: unexposed and exposed to natural gas (NG: native grass, AA: alfalfa and JM: Japanese millet).

3.2. Gas Concentration Profiles

The pore gas concentrations of N₂, O₂, CH₄, and CO₂ in the root zone at depths of 10, 20, and 30 cm from the top surface in each column are presented in Figure 5a–c, representing day 7, day 41, and day 65 at loading rates of 75.1 gCH₄/m²/d, 437.9 gCH₄/m²/d, and 845.2 gCH₄/m²/d, respectively. These results indicate that the columns with native grass (NG) and Japanese millet (JM) contained less N₂ and O₂ than the column with alfalfa (AA). N₂ and O₂ concentrations along the depth are indicative of the level of soil aeration and air penetration [8], as well as the displacement of N₂ and O₂ by CH₄ gas fed from the bottom

of the columns. Furthermore, O₂ consumption by methanotrophic bacteria will impact the O₂ profiles. Whalen et al. pointed out that root morphology might play a major role in determining air penetration [36]. Root systems in alfalfa consist of deep taproots, while the root structures in native grass (and Japanese millet to a lesser extent) are shallow and fibrous. Such changes and root structure, as well as the differences in the rates of methane oxidation, may explain the distinct difference in gas profiles observed amongst the three vegetation species.

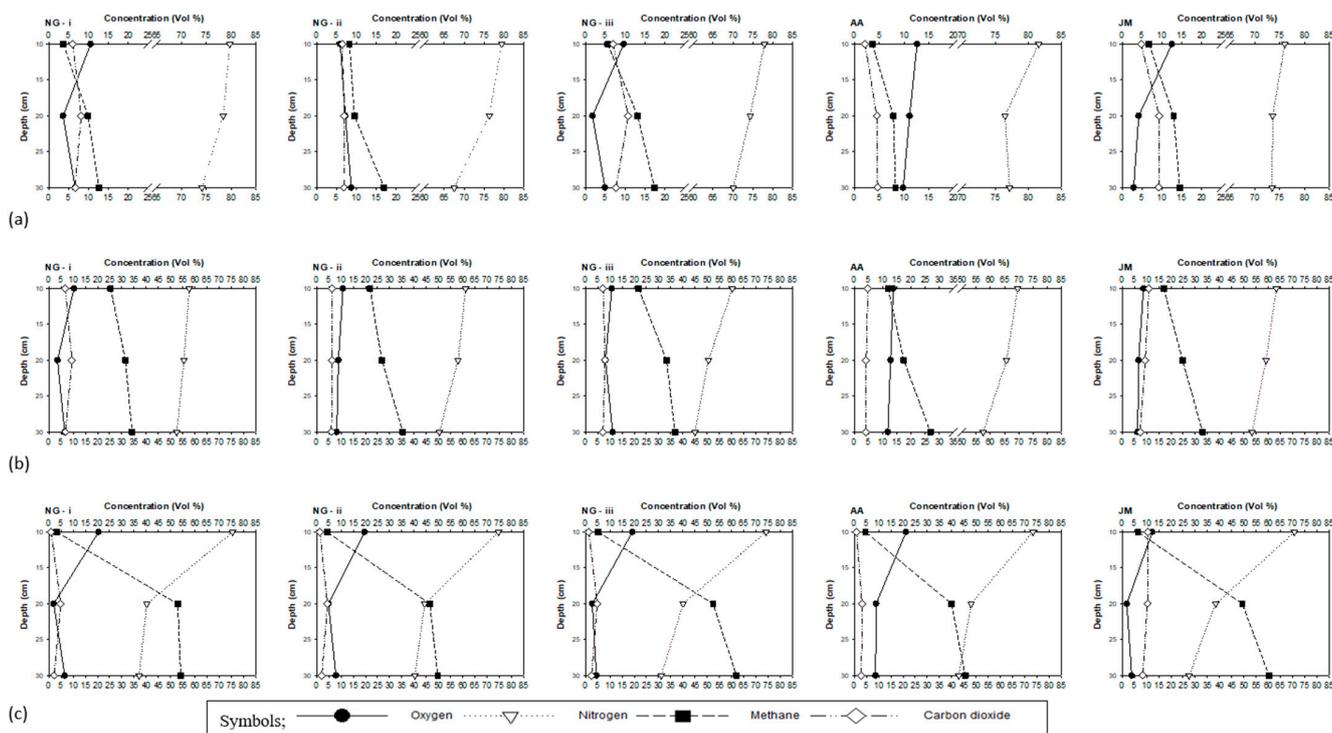


Figure 5. Depth profiles of the columns exposed to gas: NG-i, NG-ii, and NG-iii (native grass triplicates), AA—alfalfa, JM—Japanese millet, at different CH₄ loading rates. (a) CH₄ loading rate of 75.1 gCH₄/m²/d on day 7, (b) CH₄ loading rate of 437.9 gCH₄/m²/d on day 41, (c) CH₄ loading rate of 845.2 gCH₄/m²/d on day 65.

The highest concentrations of N₂ among the gas-exposed columns were observed in column AA (or the column vegetated with alfalfa consisting of deep taproots). The values ranged from 81.5% to 77.2%, 69.6% to 57.5%, and 73.3% to 42.8% at loading rates of 75.1 gCH₄/m²/d, 437.9 gCH₄/m²/d, and 845.2 gCH₄/m²/d, respectively, demonstrating that the displacement of N₂ increases with increasing loading rates. Lower N₂ concentrations were observed in JM and NG columns. The lowest N₂ concentrations were observed in JM column at a loading rate of 845.2 gCH₄/m²/d, with 70.6% at 10 cm, 38.4% at 20 cm, and 27.4% at 30 cm. The O₂ concentrations in columns followed a similar trend in general, with AA column exhibiting O₂ concentration values ranging from 12.6% to 9.8%, 13.7% to 11.8%, and 21.2% to 8.7% at loading rates of 75.1 gCH₄/m²/d, 437.9 gCH₄/m²/d, and 845.2 gCH₄/m²/d, respectively. Similar to N₂ concentrations, the lowest O₂ concentrations were observed in JM column at a loading rate of 845.2 gCH₄/m²/d, with 12.3%, 1.9%, and 3.9% at depths 10, 20, and 30 cm, respectively. The results from this study are similar to those reported by Whalen et al. [36]. They reported that lysimeters with grass had lower aeration, indicated by low O₂, than a lysimeter with alfalfa and grass, indicated by high O₂ levels.

The CO₂ profiles showed a different trend simply because CO₂ concentrations are not directly impacted by root morphology and air penetration, but by the level of root respiration (if any) and methane oxidation occurring in the root zone. At the loading rate of

75.1 gCH₄/m²/d, the highest concentrations of CO₂ were observed in column NG-iii with concentrations of 6.9%, 10.8%, and 7.7%, at depths 10, 20, and 30 cm, respectively. These concentration results are indicative of the occurrence of methane oxidation in the 20 cm depth range. Typically, the majority of methane oxidation in columns occurs at depths between 10 to 30 cm below the column surface. The CO₂ concentrations in column AA were low, with concentrations ranging from 2.1% to 4.7%, 3.9% to 4.8%, and 0.9% to 3.1% at loading rates of 75.1 gCH₄/m²/d, 437.9 gCH₄/m²/d, and 845.2 gCH₄/m²/d, respectively. In column JM, at a loading rate of 845.2 gCH₄/m²/d, the CO₂ concentrations were 10.5%, 10.4%, and 8.4%, each at depths of 10, 20, and 30 cm, respectively.

The CH₄ concentrations in vegetated columns are determined by the loading rates and methane oxidation, with high CH₄ concentrations typically observed at a depth of 30 cm. At the lowest CH₄ loading rate of 75.1 gCH₄/m²/d, the CH₄ concentrations ranged from 3.7% to 12.6% in NG-i, 8.2% to 16.9% in NG-ii, 5.6% to 17.4% in NG-iii, 3.7% to 8.3% in AA, and 6.7% to 14.4% in JM at depths between 10 and 30 cm. During the experiment, the highest CH₄ concentration of 61.9% was observed in the natural gas column NG-iii exhibited at 30 cm depth and at the highest loading rate of 845.2 gCH₄/m²/d. Although this column showed a relatively high oxidation rate, most oxidation seemed to have taken place between the depths of 10 to 20 cm. In general, the columns vegetated with native grass showed the highest CH₄ concentrations throughout the experiment, and the lowest CH₄ concentrations were exhibited by column AA. For example, at the loading rate of 437.9 gCH₄/m²/d, the CH₄ concentration values ranged from 25.3% to 34.2% in NG-i, 21.8% to 35.3% in NG-ii, and 21.8% to 36.7% in NG-iii at depths of 10 to 30 cm, whereas, the values for column AA ranged from 11.9% to 26.8% at depths of 10 to 30 cm. This observation is attributed to alfalfa plants having deep taproots, which allows an escape of CH₄ gas from deeper parts of the columns via the macropores created by the deep roots.

Both CH₄ and CO₂ may exhibit negative impacts on the growth of a plant; CH₄, although not a phytotoxin, causes displacement of O₂ when present in high concentrations [20]. Previous studies have shown that high CO₂ concentrations in the root zone of a plant can considerably hinder its growth [37]. Roots under hypoxic stress may transmit signals to the leaves, limiting leaf growth and substrate transportation to the root system, and promoting the accumulation of certain adverse compounds in the leaves [38,39].

Root morphology might play a major role in the performance of the system: roots take up water, increasing permeability (i.e., increasing penetration of N₂ and O₂). Root systems in alfalfa consist of deep taproots that can extend to 35 cm, while the root systems in grass are shallow (root mat) and only extend to about 20 cm [24]. Alfalfa plants used in our study had taproots, while the native grass and Japanese millet had fibrous root structures, explaining the distinct difference in gas profiles amongst the different vegetation species.

3.3. Methane Oxidation in Vegetated Columns

The average CH₄ oxidation rates and oxidation efficiencies at each flux rate are shown in Table 3. The time and flux dependent oxidation rates for columns with different types of vegetation (NG—average from native grass triplicates of NG-i, NG-ii and NG-iii), AA (alfalfa), and JM (Japanese millet) are presented in Figure 6.

Although the CH₄ oxidation efficiency fluctuated, the CH₄ oxidation rate increased with an increase in CH₄ influx in all columns. The column JM, with Japanese millet, exhibited the highest methane oxidation efficiencies and oxidation rates throughout the experiment. The highest average methane oxidation efficiency was observed in column JM with a value of 47.5% at the loading rate of 285.1 gCH₄/m²/d. In column JM, CH₄ oxidation rate increased from 27.5 to 246.8 gCH₄/m²/d when the CH₄ loading rate increased from 75.1 to 845.2 gCH₄/m²/d. The corresponding methane oxidation rate values for columns AA and NG (average of triplicates) were 15.4 to 189.3 gCH₄/m²/d and 20 to 222.6 gCH₄/m²/d, respectively. The oxidation rates observed in this study were much higher than the rates observed by other researchers with vegetated columns. In a study by Bohn et al., where the overall maximum loading rate was 89.6 gCH₄/m²/d, the three vege-

tated columns showed maximum oxidation rates of 89.6 gCH₄/m²/d, 62.4 gCH₄/m²/d, and 36.8 gCH₄/m²/d for the columns with grass mixture, Canadian goldenrod, and leguminous mixture, respectively [23]. They observed an increase in O₂ diffusion into the granular medium that potentially increased methane oxidation. Similar to our study, the extended plant roots, especially JM columns vegetated with Japanese millet, may have increased the air-filled capacity and O₂ diffusion into the root zone.

Table 3. Average oxidation rate and oxidation efficiencies of columns.

Loading Rate (g/m ² /d)	Oxidation Rate (gCH ₄ /m ² /d)			Oxidation Efficiency (%)		
	NG (Average of i, ii and iii)	AA	JM	NG	AA	JM
75.1	20.0	15.4	27.5	26.6	20.5	36.6
173.1	73.2	68.4	76.1	42.3	39.5	43.9
285.1	134.8	132.3	135.4	47.3	46.4	47.5
437.9	153.2	137.5	165.5	35	31.4	37.8
635.1	222.1	186.1	250.2	34.9	29.3	39.4
845.2	222.5	189.3	246.8	26.3	22.4	29.2

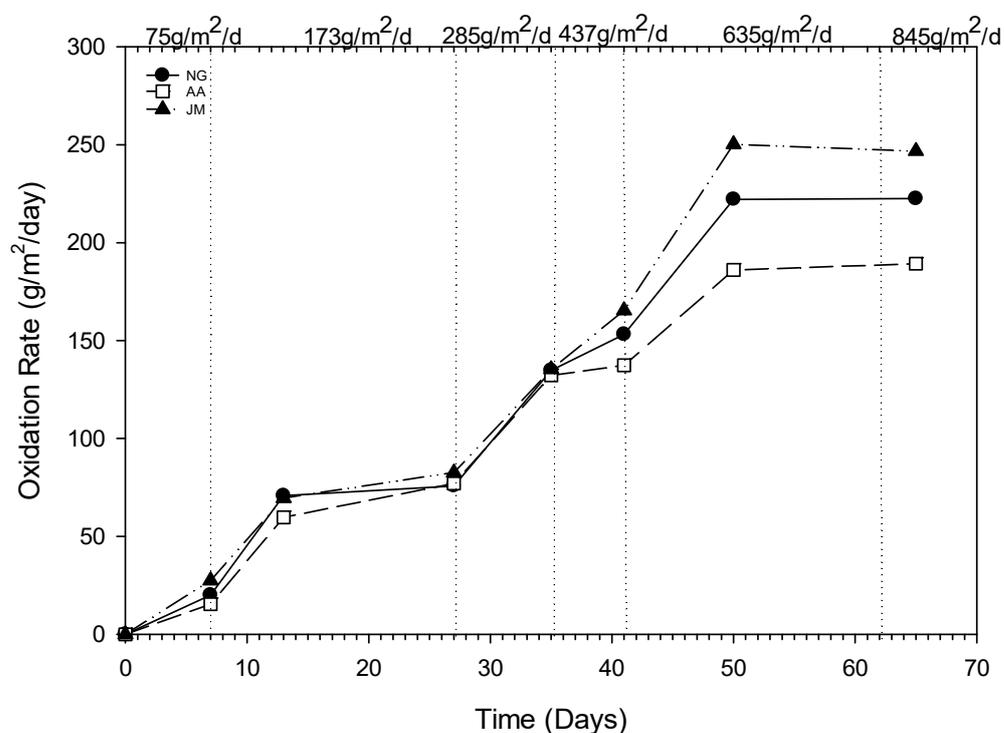


Figure 6. Time-dependent methane oxidation rates (NG: native grass (triplicates indicated as i, ii and iii), AA: alfalfa and JM: Japanese millet).

The calculated V_{\max} values related to methane oxidation in the columns are presented in Table 4. The V_{\max} values represent the viable methanotrophic bacterial populations present in the soil core samples obtained from each column on day 65. The methanotrophic activity V_{\max} values of the gas-exposed columns, from highest (6.64 $\mu\text{mol/g dw/h}$) to lowest (2.92 $\mu\text{mol/g dw/h}$), were as follows: JM > NG > AA. This data corresponds well with the calculated CH₄ oxidation rates presented in Table 3, with the highest oxidation rates being observed in JM, while the lowest were observed in AA. The V_{\max} values obtained in this study are comparable to values of 2.8 $\mu\text{mol/g dw/h}$ [40] and 7.7 $\mu\text{mol/g dw/h}$ [31] obtained in other studies conducted on the methanotrophic activities of cover soil material in the presence of CH₄ [31].

Table 4. V_{max} values for columns at depths of 15–25 cm.

Kinetic Parameter	NG	AA	JM	NG-con	AA-con	JM-con
V _{max} ($\mu\text{mol/g dw/h}$)	3.97	2.92	6.64	0.22	0.24	0.51

4. Discussion and Interpretation of Results

4.1. Oxygen Gas Concentrations and Vegetation Impacts

The gas concentration profiles of columns presented in Figure 5a–c, representing exposure to the flux rates of 75.1 to 845 gCH₄/m²/d, show that O₂ concentrations in almost all vegetated columns are below 10%. Low levels of O₂ affect the oxygen-dependent reactions within plants [41]. An O₂ level above 10% in the soil-gas phase of the root zone is needed for the growth of most plants, and an O₂ level below 10% causes hypoxia in plants [19,22]. It is known that root zone hypoxia stress in plants triggers the formation of reactive oxygen species, such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide, which causes damage to DNA, membranes, and proteins [42], resulting in chlorosis, necrosis, defoliation, stunted growth, and root damage [41,43]. Plant cells under hypoxic conditions synthesize higher concentrations of ethylene, a plant hormone, which then induces the formation of lysigenous aerenchyma—gas filled spaces resulting from lysis and the death of cells in the root cortex [44,45].

In the current study, the lowest O₂ concentrations were observed with native grass columns (average of NG-i, NG-ii and NG-iii), and consequently, these columns exhibited the highest impact on vegetation. For example, at the relatively high flux rate of 845 gCH₄/m²/d, the O₂ concentrations in the 20 cm region were well below 5%, and there was a large reduction of 51% in plant height and 35% in root length in the native grass blend in comparison with the plants in the control column. The corresponding values for Japanese millet were a 31.5% reduction in plant height and 25% in root length, and in alfalfa were a 19% reduction in plant height and 17% in root length. Whalen et al. [36] pointed out that root morphology might play a major role in the plant behavior: roots take up water, increasing permeability, thereby increasing the penetration of N₂ and O₂. Alfalfa root systems are characterized by deep taproots that can extend up to 50–60 cm, creating preferential pathways for gas, water, and nutrients. In contrast, grass root systems form a shallow root mat that only extends 20–30 cm below the surface [24]. The alfalfa plants used in our study also had taproots, whereas the native grass and Japanese millet had fibrous root structures, as depicted in Figure 7. This difference in root structures may explain the distinct differences in gas profiles observed among the different vegetation species. Some plants may undergo a physical and morphological adaptation known as “Aerenchyma formation” to overcome hypoxic conditions in soil [46,47]. Aerenchyma are a form of cells that ensure the survival of certain plants that are under extremely O₂ deficient conditions and act as conduits that supply O₂ to the roots [48,49]. It appears that Japanese millet plants have the capability to tolerate hypoxia to some extent because of the supply of O₂ to the roots via the lysigenous aerenchyma along the adventitious root [50].

4.2. Methane Oxidation and Vegetation Impacts

Methane oxidation by methanotrophs could impact plant growth in two ways; by consuming O₂ in competition with plants and by producing CO₂ that could impact plant growth. In experimental soil columns, most methanotrophic activity occurs in a narrow range about 15–40 cm below surface, known as the zone of methane oxidation [51]. However, in vegetated columns, this zone could extend beyond this range because of the changes in porosity in the presence of plant roots [10]. As a result of the presence of roots, the zone of methane oxidation is not highly pronounced in our vegetated columns. Nevertheless, the concentration profiles of columns presented in Figure 5a–c show high levels of CO₂ in the 5 to 10% range, in almost all vegetated columns. The presence of CO₂ is the result of methane oxidation by methanotrophs that converts CH₄ to CO₂ and water.

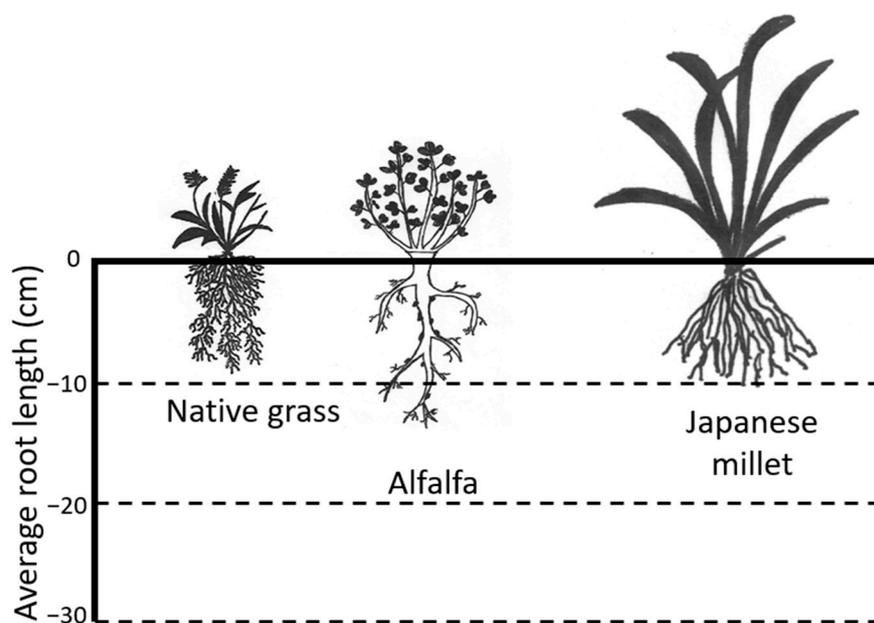


Figure 7. Schematic diagram of plants used in the study.

Past research studies have shown that when CO_2 exceeds 20% volume in the soil pore space, vegetation could be negatively impacted [18,52]. Even lower CO_2 levels of 2% (by volume) in the root zone of pea plants (*Pisum sativum* L.) have been shown to reduce the growth of the roots by 80% [37]. Considering almost all of our columns exhibited greater than 2% CO_2 (by volume), it is not surprising to observe impacts on root growth of all plants tested.

Another consequence of methane oxidation in columns is the consumption of O_2 in the root zone by methanotrophs decreasing the pore O_2 available for plants. As expected, CH_4 oxidation rates increased with an increase in CH_4 influx, and therefore, the highest oxidation rates were observed at the highest methane flux rate of $845 \text{ gCH}_4/\text{m}^2/\text{d}$. The columns with Japanese millet exhibited the highest methane oxidation rate of $250.2 \text{ gCH}_4/\text{m}^2/\text{d}$. The corresponding methane oxidation rates were $222.5 \text{ gCH}_4/\text{m}^2/\text{d}$ for column NG, and $189.3 \text{ gCH}_4/\text{m}^2/\text{d}$ for alfalfa. Since these values were within the experimental error for the column experiments, definitive conclusions cannot be made as to the presence of a direct correlation between methane oxidation rate and the occurrence of hypoxia in columns, which is the primary cause of vegetation impacts. Furthermore, we observed cumulative negative impacts on vegetation from the reduction of O_2 in the root zone, as well as the increase in CO_2 levels because of methane oxidation. One other confounding factor is the increased diffusion into the root zone of O_2 from the atmosphere because of the macropores created by the growth of root systems over time [10]. This phenomenon of increased O_2 diffusion into the root zone tends to counterbalance the decrease in O_2 because of methane oxidation and high loading rates of gas.

5. Conclusions

Considering past research that has shown vegetation on landfills could be impacted because of potential hypoxia in instances where (1) generated landfill gas displaces oxygen in the root zone of the plants, and (2) plants compete for oxygen with methanotrophic bacteria present in the root zone, this study was conducted to investigate the effects of methane exposure on three types of potential landfill vegetation, native grass, alfalfa, and Japanese millet. The study included conducting experiments outdoors to determine time-dependent changes in gas concentration profiles, methane oxidation rates, and efficiencies and kinetics, as well the determination of changes to plant height, root length, dry mass density, and chlorophyll content during exposure to methane flux rates ranging from $75 \text{ gCH}_4/\text{m}^2/\text{d}$

to 845 gCH₄/m²/d. Although definitive results could not be obtained due to constraints associated with conducting outdoor experiments under semi-controlled conditions, practical implications of the experimental results were clear. Plant growth in landfill biocover systems will be somewhat impacted even at 75 gCH₄/m²/d of methane fluxes, representative of typical average flux rates expected at waste cells accepting biodegradable organic waste. At higher flux rates representative of hotspots on a landfill surface, the impacts will be greater, but extremely high flux rates, greater than 845 gCH₄/m²/d, are required to completely kill off vegetation.

Author Contributions: Conceptualization, D.S.A. and J.P.A.H.; methodology, D.S.A.; validation, D.S.A., J.P.A.H. and A.C.; formal analysis, D.S.A.; data curation, D.S.A.; writing—original draft preparation, D.S.A.; writing—review and editing, D.S.A., J.P.A.H. and P.A.J.; visualization, D.S.A. and D.P.; supervision, J.P.A.H.; project administration, J.P.A.H.; funding acquisition, J.P.A.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Natural Sciences and Engineering Research Council of Canada-Collaborative Research and Development (NSERC—CRD) grant number [RGPIN-2012-122174].

Data Availability Statement: All data, models, or code that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments: Financial support for this study was provided by Centre for Environmental Engineering Research and Education (CEERE) at the University of Calgary and Natural Sciences and Engineering Research Council (NSERC) of Canada. The authors wish to thank Marcus Samuel, Eranda Bartholameuz, Hiva Jalilzadeh, Erfan Irandoost, Saheli Rao, Abhinandan Kumar and Mathew Hamilton for providing guidance and assistance to complete this research.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Hunte, C.; Hettiaratchi, J.P.A.; Hettiarachchi, H.; Meegoda, J.N. *Determination of Waste Properties from Settlement Behaviour of a Full Scale Waste Cell Operated as a Landfill Bioreactor*; ASCE GeoFrontiers: Dallas, TX, USA, 2011.
- Xiaoli, C.; Xin, Z.; Ziyang, L.; Shimaoka, T.; Nakayama, H.; Xianyan, C.; Youcai, Z. Characteristics of vegetation and its relationship with landfill gas in closed landfill. *Biomass Bioenergy* **2011**, *35*, 1295–1301. [[CrossRef](#)]
- Bogner, J.E.; Spokas, K.A.; Burton, E.A. Temporal variations in greenhouse gas emissions at a midlatitude landfill. *J. Environ. Qual.* **1999**, *28*, 278–288. [[CrossRef](#)]
- Kightley, D.; Nedwell, D.B.; Cooper, M. Capacity for methane oxidation in landfill cover soils measured in laboratory-scale soil microcosms. *Appl. Environ. Microbiol.* **1995**, *61*, 592–601. [[CrossRef](#)]
- La, H.; Hettiaratchi, J.P.A.; Achari, G.; Verbeke, T.J.; Dunfield, P.F. Biofiltration of methane using hybrid mixtures of biochar, lava rock and compost. *Environ. Pollut.* **2018**, *241*, 45–54. [[CrossRef](#)]
- Tao, Z.; Shi, W.; Liu, Y.; Chai, X. Temporal variation of vegetation at two operating landfills and its implications for landfill phytoremediation. *Environ. Technol.* **2020**, *41*, 649–657. [[CrossRef](#)]
- Khapre, A.; Kumar, S.; Rajasekaran, C. Phytocapping: An alternate cover option for municipal solid waste landfills. *Environ. Technol.* **2019**, *40*, 2242–2249. [[CrossRef](#)]
- Reichenauer, T.G.; Watzinger, A.; Riesing, J.; Gerzabek, M.H. Impact of different plants on the gas profile of a landfill cover. *Waste Manag.* **2011**, *31*, 843–853. [[CrossRef](#)]
- Stralis-Pavese, N.; Sessitsch, A.; Weilharter, A.; Reichenauer, T.; Riesing, J.; Csontos, J.; Murrell, J.C.; Bodrossy, L. Optimization of diagnostic microarray for application in analysing landfill methanotroph communities under different plant covers. *Environ. Microbiol.* **2004**, *6*, 347–363. [[CrossRef](#)]
- Attalage, D.S.; Hettiaratchi, P.A.; Jayasinghe, P.; Dunfield, P.F.; Smirnova, A.V.; Rathnavibushana, U.K.; Erkmén, M.; Kumar, S. Field study on the effect of vegetation on the performance of soil methanotrophy-based engineered systems—Column experiments. *Soil Biol. Biochem.* **2022**, *167*, 108583. [[CrossRef](#)]
- Reay, D.S.; Nedwell, D.B.; McNamara, N.; Ineson, P. Effect of tree species on methane and ammonium oxidation capacity in forest soils. *Soil Biol. Biochem.* **2005**, *37*, 719–730. [[CrossRef](#)]
- Chan, Y.S.G.; Chu, L.M.; Wong, M.H. Influence of landfill factors on plants and soil fauna—An ecological perspective. *Environ. Pollut.* **1997**, *97*, 39–44. [[CrossRef](#)] [[PubMed](#)]
- Wong, M.H.; Yu, C.T. Monitoring of gin drinkers' bay landfill, Hong Kong: II. Gas contents, soil properties, and vegetation performance on the side slope. *Environ. Manag.* **1989**, *13*, 753–762. [[CrossRef](#)]

14. Wong, M.H.; Yu, C.T. Monitoring of gin drinkers' bay landfill, Hong Kong: I. landfill gas on top of the landfill. *Environ. Manag.* **1989**, *13*, 743–752. [[CrossRef](#)]
15. Leone, I.A.; Flower, F.B.; Arthur, J.J.; Gilman, E.F. Damage to New Jersey crops by landfill gases. *Plant Dis. Rep.* **1977**, *61*, 295–299.
16. Gilman, E.F.; Leone, I.A.; Flower, F.B. The adaptability of 19 woody species in vegetating a former sanitary landfill. *For. Sci.* **1981**, *27*, 13–18.
17. Wong, M.H.; Cheung, Y.H.; Cheung, C.L. The effects of ammonia and ethylene oxide in animal manure and sewage sludge on the seed germination and root elongation of brassica parachinensis. *Environ. Pollution. Ser. A Ecol. Biol.* **1983**, *30*, 109–123. [[CrossRef](#)]
18. Gendebien, A.; Pauwels, M.; Ledrut-Damanet, M.J.; Nyns, E.J.; Willumsen, H.C.; Butson, J.; Fabry, R.; Ferrero, G.L. Potential Landfill Gas Damages to Vegetation. In *Landfill Gas from Environment to Energy*; Commission of the European Communities: Luxembourg, 1992; pp. 35–46.
19. Kozłowski, T.T. Soil aeration, flooding, and tree growth. *J. Arboric.* **1985**, *11*, 85–96. [[CrossRef](#)]
20. Nagendran, R.; Selvam, A.; Joseph, K.; Chiemchaisri, C. Phytoremediation and rehabilitation of municipal solid waste landfills and dumpsites: A brief review. *Waste Manag.* **2006**, *26*, 1357–1369. [[CrossRef](#)]
21. Gilman, E.F.; Leone, I.A.; Flower, F.B. Influence of soil gas contamination on tree root growth. *Plant Soil* **1982**, *65*, 3–10. [[CrossRef](#)]
22. Danielson, R.E. Physical edaphology—The physics of irrigated and nonirrigated soils. *Soil Sci. Soc. Am. J.* **1974**, *38*, iv. [[CrossRef](#)]
23. Bohn, S.; Brunke, P.; Gebert, J.; Jager, J. Improving the aeration of critical fine-grained landfill top cover material by vegetation to increase the microbial methane oxidation efficiency. *Waste Manag.* **2011**, *31*, 854–863. [[CrossRef](#)] [[PubMed](#)]
24. Jalilzadeh, H. Field Performance and Water Balance Predictions of Evapotranspirative Landfill Biocovers. Master's Thesis, University of Calgary, Calgary, AB, Canada, 2019.
25. Adenipekun, C.O.; Oyetunji, O.J.; Kassim, L.S. Effect of spent engine oil on the growth parameters and chlorophyll content of corchorus olitorius linn. *Environmentalist* **2008**, *28*, 446–450. [[CrossRef](#)]
26. Cornelissen, J.H.C.; Lavorel, S.; Garnier, E.; Díaz, S.; Buchmann, N.; Gurvich, D.E.; Reich, P.B.; ter Steege, H.; Morgan, H.D.; van der Heijden, M.G.A.; et al. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Aust. J. Bot.* **2003**, *51*, 335–380. [[CrossRef](#)]
27. Singh, S.N.; Rao, D.N. Certain responses of wheat plants to cement dust pollution. *Environ. Pollut. Ser. A Ecol. Biol.* **1981**, *24*, 75–81. [[CrossRef](#)]
28. Maclachlan, S.; Zalik, S. Plastid structure, chlorophyll concentration, and free amino acid composition of a chlorophyll mutant of barley. *Can. J. Bot.* **1963**, *41*, 1053–1062. [[CrossRef](#)]
29. Pihlatie, M.K.; Christiansen, J.R.; Aaltonen, H.; Korhonen, J.F.J.; Nordbo, A.; Rasilo, T.; Benanti, G.; Giebels, M.; Helmy, M.; Sheehy, J.; et al. Comparison of static chambers to measure CH₄ emissions from soils. *Agric. Meteorol.* **2013**, *171–172*, 124–136. [[CrossRef](#)]
30. Powelson, D.K.; Chanton, J.; Abichou, T.; Morales, J. Methane oxidation in water-spreading and compost biofilters. *Waste Manag. Res. J. A Sustain. Circ. Econ.* **2006**, *24*, 528–536. [[CrossRef](#)]
31. Pokhrel, D.; Hettiaratchi, J.; Steele, M. Methane oxidation prediction curves of soil at different organic contents. *Curr. Environ. Manag.* **2016**, *3*, 131–143. [[CrossRef](#)]
32. Pokhrel, D. Compost Based Biocap Performance. Ph.D. Thesis, University of Calgary, Calgary, AB, Canada, 2006.
33. Flower, F.B.; Gilman, E.F.; Leone, I.A. Landfill gas, what it does to trees and how its injurious effects may be prevented. *J. Arboric.* **1981**, *7*, 43–52. [[CrossRef](#)]
34. Mi, Y.; Ma, X.; Chen, S. Resistant evaluation of kiwifruit rootstocks to root zone hypoxia stress. *Am. J. Plant Sci.* **2013**, *4*, 945–954. [[CrossRef](#)]
35. Cao, F.L.; Conner, W.H. Selection of flood-tolerant populus deltoides clones for reforestation projects in China. *For. Ecol. Manag.* **1999**, *117*, 211–220. [[CrossRef](#)]
36. Whalen, S.C.; Reeburgh, W.S.; Sandbeck, K.A. Rapid methane oxidation in a landfill cover soil. *Appl. Environ. Microbiol.* **1990**, *56*, 3405–3411. [[CrossRef](#)]
37. Stolwijk, J.A.J.; Thimann, K.V. On the uptake of carbon dioxide and bicarbonate by roots, and its influence on growth. *Plant Physiol.* **1957**, *32*, 513–520. [[CrossRef](#)] [[PubMed](#)]
38. Jackson, M.B.; Hall, K.C. Early stomatal closure in waterlogged pea plants is mediated by abscisic acid in the absence of foliar water deficits. *Plant Cell Environ.* **1987**, *10*, 121–130. [[CrossRef](#)]
39. Milligan, S.P.; Dale, J.E. The effects of root treatments on growth of the primary leaves of phaseolus Vulgaris L.: General features. *N. Phytol.* **1988**, *108*, 27–35. [[CrossRef](#)]
40. Chiemchaisri, W.; Visvanathan, C.; Wu, J.S. Biological activities of methane oxidation in tropical landfill cover soils. *J. Solid Waste Technol. Manag.* **2001**, *27*, 129–136.
41. Hanslin, H.M.; Sæbø, A.; Bergersen, O. Estimation of oxygen concentration in the soil gas phase beneath compost mulch by means of a simple method. *Urban For. Urban Green* **2005**, *4*, 37–40. [[CrossRef](#)]
42. Geigenberger, P. Response of plant metabolism to too little oxygen. *Curr. Opin. Plant Biol.* **2003**, *6*, 247–256. [[CrossRef](#)]
43. Boru, G.; Vantoai, T.; Alves, J.; Hua, D.; Knee, M. Responses of soybean to oxygen deficiency and elevated root-zone carbon dioxide concentration. *Ann. Bot.* **2003**, *91*, 447–453. [[CrossRef](#)]
44. Drew, M.C. Oxygen deficiency and root metabolism: Injury and acclimation under hypoxia and anoxia. *Annu. Rev. Plant Biol.* **1997**, *48*, 223–250. [[CrossRef](#)] [[PubMed](#)]
45. Evans, D.E. Aerenchyma Formation. *N. Phytol.* **2004**, *161*, 35–49. [[CrossRef](#)]

46. Joshi, R.; Kumar, P. Lysigenous aerenchyma formation involves non-apoptotic programmed cell death in rice (*Oryza Sativa* L.) roots. *Physiol. Mol. Biol. Plants* **2012**, *18*, 1–9. [[CrossRef](#)] [[PubMed](#)]
47. Jiang, Z.; Song, X.F.; Zhou, Z.Q.; Wang, L.K.; Li, J.W.; Deng, X.Y.; Fan, H.Y. Aerenchyma formation: Programmed cell death in adventitious roots of winter wheat (*Triticum aestivum*) under waterlogging. *Funct. Plant Biol.* **2010**, *37*, 748–755. [[CrossRef](#)]
48. Colmer, T.D. Long-distance transport of gases in plants: A perspective on internal aeration and radial oxygen loss from roots. *Plant Cell Environ.* **2003**, *26*, 17–36. [[CrossRef](#)]
49. Yamauchi, T.; Tanaka, A.; Mori, H.; Takamura, I.; Kato, K.; Nakazono, M. Ethylene-dependent aerenchyma formation in adventitious roots is regulated differently in rice and maize. *Plant Cell Environ.* **2016**, *39*, 2145–2157. [[CrossRef](#)] [[PubMed](#)]
50. Matsuura, A.; Kato, Y.; Suzuki, T.; Murata, K.; An, P. Hypoxia tolerance of four millet species is attributable to constitutive aerenchyma formation and root hair development of adventitious roots. *Plant Prod. Sci.* **2022**, *25*, 157–171. [[CrossRef](#)]
51. Visvanathan, C.; Pokhrel, D.; Cheimchaisri, W.; Hettiaratchi, J.P.A.; Wu, J.S. Methanotrophic activities in tropical landfill cover soils: Effects of temperature, moisture content and methane concentration. *Waste Manag. Res.* **1999**, *17*, 313–323. [[CrossRef](#)]
52. Ponnampuruma, F.N. The chemistry of submerged soils. *Adv. Agron.* **1972**, *24*, 29–96. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.