



RESEARCH ARTICLE

# Effect of traffic stress and planting density on biomass and carbon exchange in bermudagrass

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## Abstract

Turfgrass often suffers from traffic stress, which reduces its biomass, including leaf wear and soil compaction. Although plant biomass is related to carbon balance over the canopy, the reduction in biomass due to traffic stress on turfgrass has not been discussed in terms of the carbon balance on turfgrass. Leaf wear can damage the photosynthetic rates of leaves and soil compaction can damage the respiration process of roots. Photosynthesis and respiration are important physiological properties, and the difference between them represents the total carbon gain over the turfgrass. This study conducted four levels of traffic experiments on bermudagrass grown in two levels of planting density, and investigated leaf and root biomass and carbon exchange, including photosynthesis and respiration. A decrease in leaf and root mass was found with the intensity of traffic stress. Photosynthetic rates per leaf mass did not change with the intensity of traffic stress but were 1.7 times higher in high planting density. On the other hand, respiration rates per plant mass decreased with the intensity of traffic stress and were 1.3 times higher in high planting density. When summarizing photosynthesis, respiration, and biomass, the total carbon balance in high-traffic intensity and low-planting density should be small. Therefore, the turfgrass population may degrade under such conditions. It can be concluded that the carbon balance over the turfgrass may serve as an indicator for detecting the effect of traffic stress and suggest management practices that take into account traffic stress and planting density, such as increasing planting density where traffic stress is high.

**Keywords:** bermudagrass; leaf and root mass; photosynthesis; planting density; traffic stress

## Introduction

Turfgrass (Cynodonteae) is widely used for its aesthetic appeal, erosion control, and recreational purposes. Turfgrass planted in sports fields or recreational areas is often subjected to traffic stress (1). Numerous studies have examined the damage to turfgrass by traffic stresses, resulting in reduced biomass (2, 3). In general, traffic stress encompasses both leaf wear and soil compaction (4). Leaf wear refers to the damage caused by direct pressure, resulting in physical harm to plant tissue. Leaves that are injured by wear lose water, leading to a decline in leaf biomass (5). Soil compaction significantly impacts the physical properties of the soil, leading to a reduction in the growth of turfgrass roots. This reduction occurs due to changes in the morphology and anatomy of the roots, which are influenced by decreased water capacity and gas exchange rates (6).

Changes in photosynthetic rates and respiration rates influence further growth of plants because accumulated carbohydrates from photosynthesis are utilized for plant growth (7, 8). Reductions in leaf quality are linked to changes in the physiological properties affecting photosynthetic rates (9). Therefore, it is essential

to monitor the carbon dynamics of the turfgrass to understand how biomass changes following physical damage. Some studies have investigated carbon exchange in turfgrass for detecting the CO<sub>2</sub> uptake ability of turfgrass (10, 11). However, carbon exchange related to physical damage has been rarely investigated. Physical damage to plant cells and environmental stresses can lead to a decrease in photosynthetic and respiration rates. Although photosynthetic rates have not been coupled with the wear stress, decreases in chlorophyll content and non-structural carbohydrates due to wear stress can be related to decreases in photosynthetic rates (12). Consequently, leaf wear and soil compaction can induce physiological changes in turfgrass associated with the plant biomass.

The dense vegetation cover acts as a natural buffer, providing protection against mechanical stresses and mitigating soil compaction, thereby safeguarding the soil properties (13). Increasing the density of the grassland may help to evenly distribute pressure on the soil surface, potentially reducing soil compaction. However, the intricate interplay between turfgrass density and the intensity of soil compaction and their combined effects on turfgrass biomass has not been extensively examined.

In this study, we aim to explore the impact of soil compaction caused by planting density and compaction intensity on the growth of turfgrass. Additionally, we seek to understand the alterations in the photosynthesis and respiration processes associated with these changes.

## Materials and Methods

### Planting procedure

This study was conducted in the nursery of the Faculty of Agriculture, Yamagata University, Japan (39°44'N, 139°82'E). We used twenty-four growing boxes (825 mm long, 525 mm wide, and 149 mm high) and each combination of two levels of planting intensity and four levels of compaction intensity was repeated three times. To prevent water retention, each growing box was drilled with 160 holes with a diameter of 6 mm in the bottom. In order to accurately measure plant respiration, it was necessary to suppress the respiration of organic matter decomposition in the soil. To achieve this, the organic matter in the soil was eliminated by burning the granite soil at 500 °C for 5 hr in a muffle furnace. It is reported that all organic matter was burnt at 400 °C for 4 hr in a muffle furnace (14), but higher temperature was given in this study to burn organic matter more steadily. Ten g of urea as nitrogen nutrients was uniformly added to the soil in each grow box. The soil was then mixed thoroughly to ensure uniformity. For this study, Riviera bermudagrass (*Cynodon dactylon* L. Pers, Takii Seed Co. Ltd, Kyoto, Japan) was used. In the high-density treatment, 60 g of seed were uniformly sown per growing box, while in the low-density treatment, 15 g of seed per growing box were used. The sowing was done using a sieve in June. Each day, the soil in the growing boxes was provided with sufficient water to ensure that water seeped out of the bottom holes.

### Traffic treatments

Once the grasses had reached their maximum height in August, we conducted the traffic treatments every two days. Traffic treatments were applied to the turf by rolling a trolley with a 10 kg load over the turf, so that pressure was applied evenly across the entire turf in the box. We applied four types of traffic treatments: 0, 5, 15, and 30 times compaction in a day. Soil hardness was measured using a Yamanaka soil hardness meter (Fujiwara Scientific Co. Ltd, Tsukuba, Japan) at 10 locations per growing box. These measurements were taken just before the traffic treatments on the day of the first compaction treatment and 2, 6, 10 and 16 days after the first treatment. Overall, measurements of soil hardness and traffic treatments were conducted eight times.

### Sampling procedures

Two days after the eighth traffic treatment, we sampled turfgrass with soil at four locations per growing box using a soil core sampler with 100 mL volume. Sampled turfgrass was inserted in an acrylic chamber connected to an infrared gas analyzer (GMP343, Vaisala, Helsinki, Sweden) and an air pump with a polyethylene tube. To detect photosynthetic capacity under full-light conditions and respiration rates, we have to measure CO<sub>2</sub> exchange rates under light and dark conditions (15). Carbon exchange rates under light and dark

conditions were calculated by the changes in CO<sub>2</sub> concentration inside the chamber under the LED light source and under a shading plastic box covered with aluminium tape, respectively. The photosynthetic photon flux density under the LED light source and under the shading box were 945  $\mu\text{mol m}^{-2}\text{s}^{-1}$  and 0  $\mu\text{mol m}^{-2}\text{s}^{-1}$ , measured using LI-190SA (Li-cor, Lincoln, Nebraska, USA). CO<sub>2</sub> exchange rates ( $\mu\text{mol s}^{-1}$ ) are calculated as the following function with the changes in CO<sub>2</sub> concentration ( $\Delta\text{CO}_2/\Delta t$ , ppm  $\text{s}^{-1}$ ), atmospheric pressure (P,  $1.013 \times 10^5$  Pa), gas constant (R,  $8.314 \text{ J K}^{-1} \text{ mol}^{-1}$ ), air temperature inside the chamber (T, °C) and chamber volume (V,  $9.456 \times 10^{-4} \text{ m}^3$ ).

$$\text{CO}_2 \text{ exchange rate} =$$

$$\frac{\Delta\text{CO}_2}{\Delta t} \cdot \frac{P \times V}{R \times (T+273.15)} \quad (\text{Eqn. 1})$$

The above ground part of the turfgrass was cut carefully so as not to disrupt the soil structure, and leaves were classified into living and dead leaves. Living and dead leaves were oven-dried at 65 °C for 48 hr and dry masses were measured. Current-year culms in bermudagrasses were small and all above-ground parts were classified as leaves, without separating leaves and culms.

The below ground part of the turfgrass with soil core sampler was put on a pan filled with water overnight to saturate the soil pores with water. Permeability in soil was quantified by measuring saturated hydraulic conductivity (16). The saturated hydraulic conductivities (kh,  $\text{cm s}^{-1}$ ) of the soil including roots were measured by the constant head permeability test using DIK-4000 (Daiki, Saitama, Japan) and calculated as the following function with changes in the volume of passing water ( $\Delta Q/\Delta t$ ,  $\text{cm}^3 \text{ s}^{-1}$ ), length of the soil column (L, 5.1 cm), sample cross-section area (A, 19.6  $\text{cm}^2$ ), and height difference of water level (H, 6.8 cm).

$$kh = \frac{\Delta Q}{\Delta t} \cdot \frac{L}{A \times H} \quad (\text{Eqn. 2})$$

Living roots were collected from the soil and dry masses were measured after oven drying at 65 °C for 48 hr.

### Photosynthesis and respiration rates

Because carbon exchange rates under light conditions include respiratory carbon emissions, leaf photosynthetic rates per sample can be viewed as the inverse sign of the differences in carbon exchange rates between light and dark conditions (15). Photosynthetic rates per ground area ( $A_{\text{area}}$ ) and per leaf dry mass ( $A_{\text{mass}}$ ) are calculated as photosynthetic rates per sample divided by the area of the soil core sampler ( $1.96 \times 10^{-3} \text{ m}^2$ ) and divided by the leaf dry mass (g), respectively. Respiration rates can be viewed as carbon exchange rates per sample under dark conditions. Respiration rates per ground area ( $R_{\text{area}}$ ) and per plant dry mass ( $R_{\text{mass}}$ ) are calculated as respiration rates per sample divided by the area of the soil core sampler ( $1.96 \times 10^{-3} \text{ m}^2$ ) and divided by the leaf and root dry mass (g), respectively.

## Statistical analysis

Linear mixed models with interaction effects were used to show the relationships between soil hardness and explanatory variables (repetition of compaction treatments, intensity of the compaction, and planting density). Relationships between other variables measured after the eighth treatment (kh, living leaf mass, dead leaf mass, root mass,  $A_{area}$ ,  $A_{mass}$ ,  $R_{area}$ , and  $R_{mass}$ ) and explanatory variables (the intensity of the compaction and planting density) were analyzed by linear mixed models with interaction effects (including intensity  $\times$  density). We analyzed these linear mixed models, assumed random effects of differences in planting boxes, and used the R package “lmerTest”.

## Results

### Soil properties

Soil hardness increased with the repetition and intensity of the traffic stress (Fig. 1 & Table 1). Low-density plots show higher soil hardness than high-density plots, with the interaction of repetition and intensity of the traffic stress. The hydraulic conductivity of the soil decreased with the intensity of the traffic stress (Fig. 2). Low-density plots have higher soil hydraulic conductivity than high-density plots.

### Leaf mass and root mass

Live leaf mass decreased with the intensity of the traffic stress (Fig. 3). Despite the differences in the mass of seeds

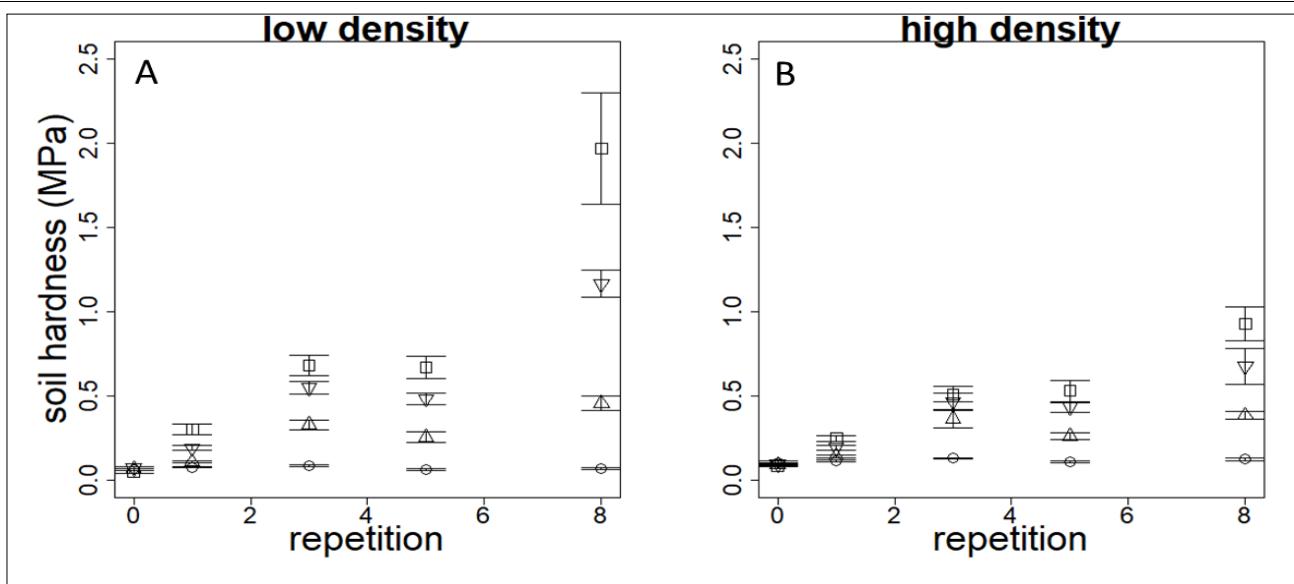
planted, there was no difference in live leaf mass between the low-density and high-density plots, especially in the plots under traffic stress. We could not mention the reason for these results, and further studies, such as density effects, are needed. There were no significant differences in live leaf mass between low-density and high-density plots. Dead leaf mass increased with the intensity of the traffic stress (Fig. 4). Dead leaf mass was also significantly influenced by the interaction of intensity of the stress and planting density, meaning that especially high dead leaf mass under high intensity of the stress in high-density plots. Root mass decreased with the intensity of the traffic stress and the planting density (Fig. 5).

### Photosynthesis and respiration

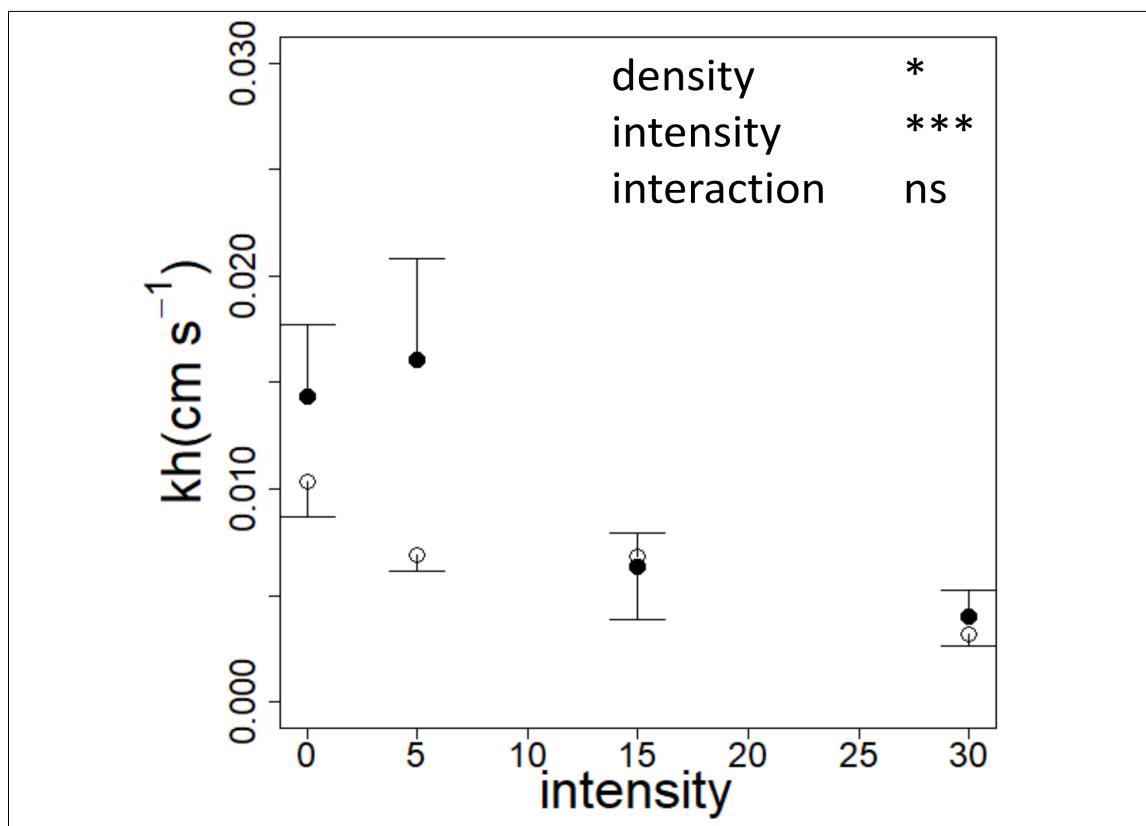
Photosynthetic rates per ground area decreased with the intensity of the traffic stress and were higher in high-density plots (Fig. 6A). Photosynthetic rates per leaf mass in high-density plots were higher than those in low-density plots (Fig. 6B). In spite of the no significant effects of intensity of the traffic stress in all samples, the intensity decreased the photosynthetic rates per leaf mass only for low-density plots. Respiration rates per ground area decreased with the intensity of the traffic stress and were higher in high-density plots (Fig. 7A). Respiration rates per total plant mass also decreased with the intensity of the traffic stress and were higher in high-density plots (Fig. 7B).

**Table 1.** Relationships between soil hardness and explanatory variables (repetition of compaction treatments, intensity of the compaction, and planting density) were shown by a linear mixed model. F and P mean F-values and P-values on the linear mixed model. \*:  $p < 0.05$ , \*\*\*:  $p < 0.001$

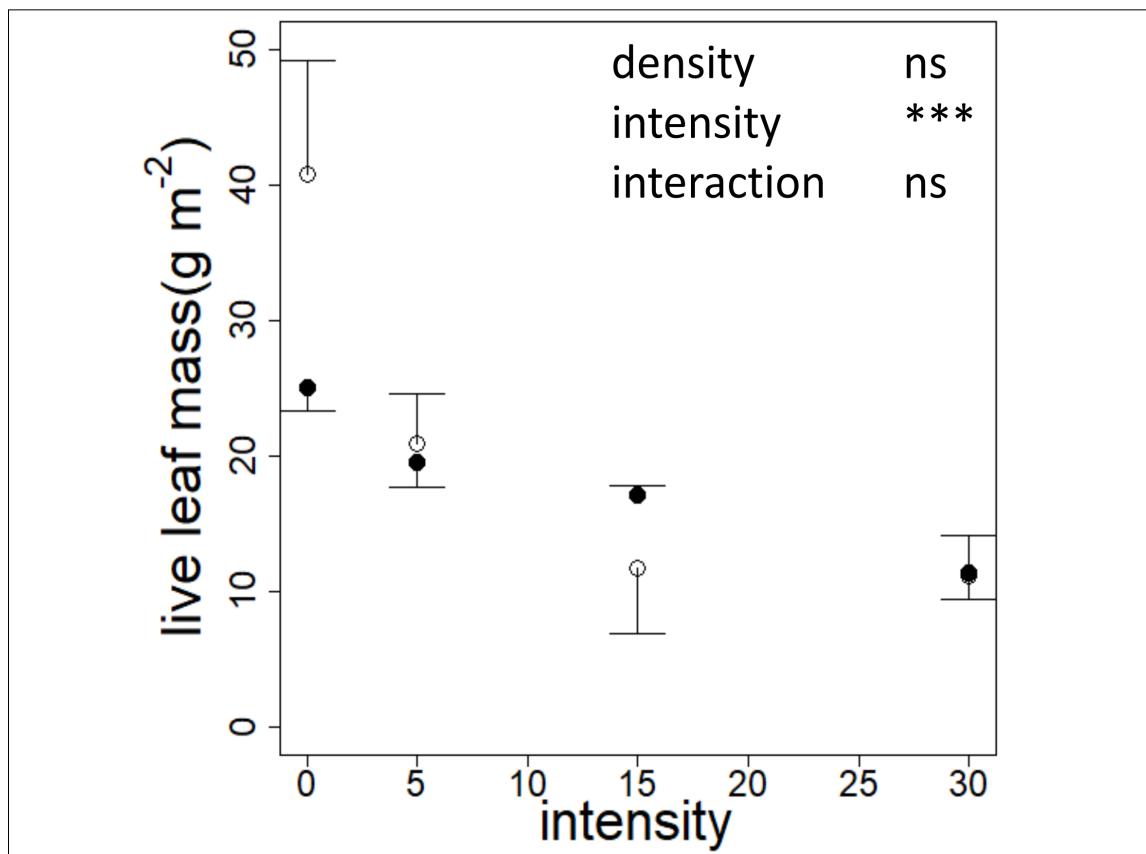
	F	P	
Repetition	53.53	<0.001	***
Intensity	4.38	0.046	*
Planting density	2.48	0.127	
Repetition $\times$ Intensity	167.24	<0.001	***
Repetition $\times$ Planting density	0.37	0.541	
Intensity $\times$ Planting density	0.39	0.536	
Repetition $\times$ Intensity $\times$ Planting density	6.41	0.012	*



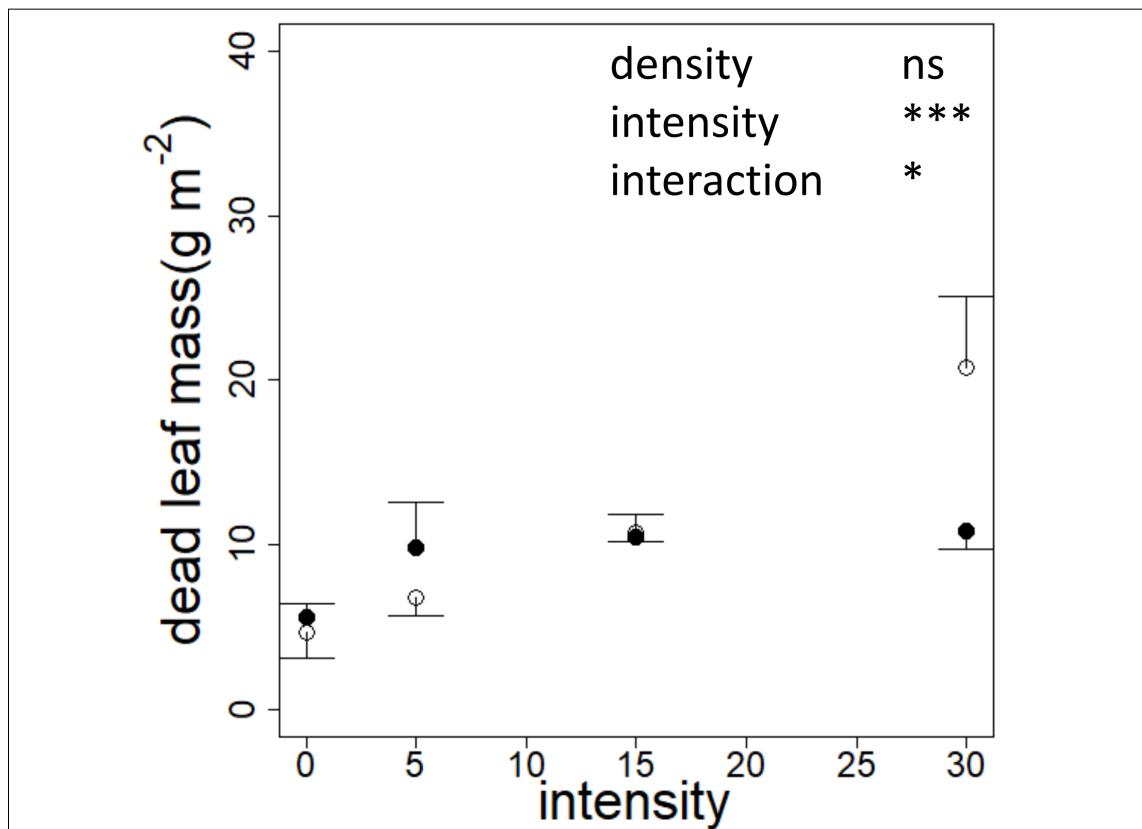
**Fig. 1.** Relationships between soil hardness and explanatory variables (repetition of compaction treatments, intensity of the compaction, and planting density). The left graph shows the soil hardness in boxes with low planting density of Bermuda grass and the right graph shows the soil hardness in boxes with high planting density of Bermuda grass. Circles, upward triangles, downward triangles, and rectangles are 0, 5, 15 and 30 times compaction in a day respectively. Results of the statistical tests are shown in Table 1.



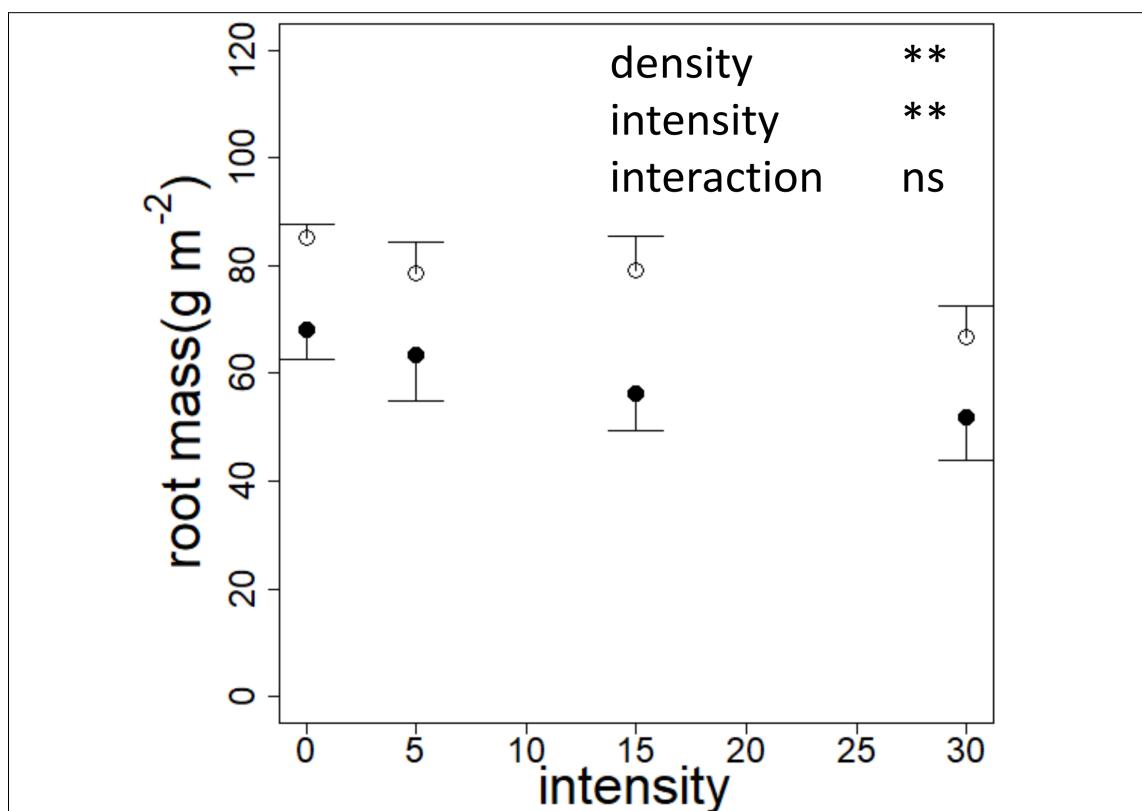
**Fig. 2.** Relationships between soil hydraulic conductivities (kh) and explanatory variables (planting density and compaction intensity) after the eighth traffic treatment. Opened circles and closed circles denote the high and low planting density of Bermuda grass respectively. The result of the statistical test by linear mixed model is also shown. “Interaction” means planting density  $\times$  treatment intensity. ns:  $p > 0.05$ , \*:  $p < 0.05$ , \*\*\*:  $p < 0.001$ .



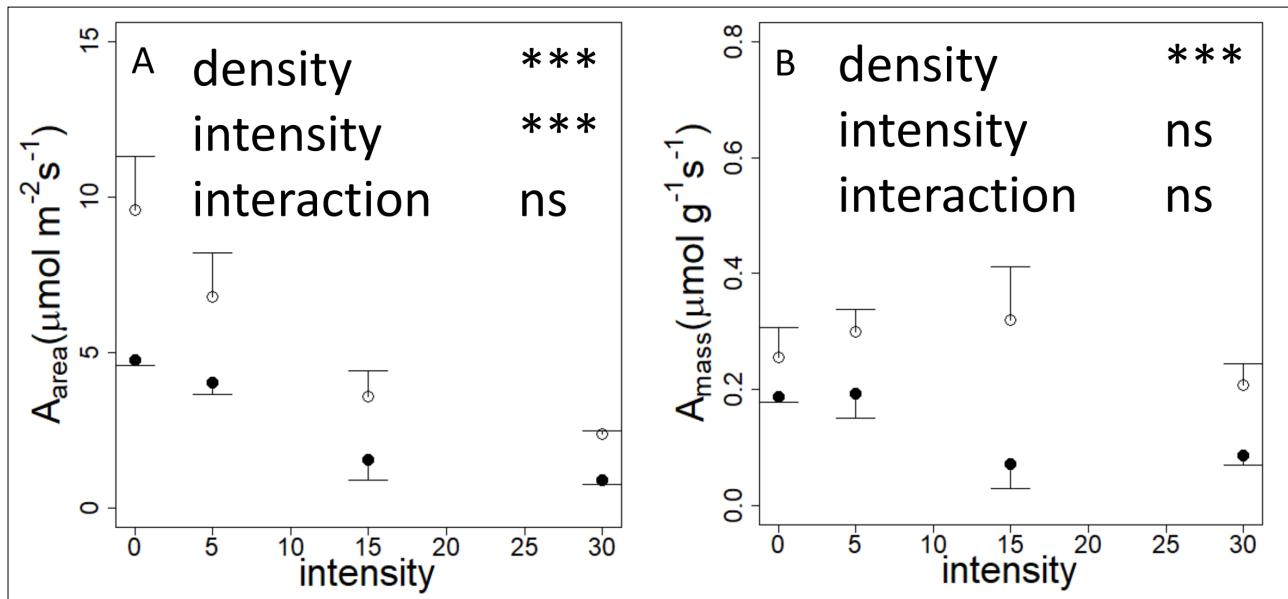
**Fig. 3.** Relationships between living leaf mass and explanatory variables (planting density and compaction intensity) after the eighth traffic treatment. Opened circles and closed circles denote the high and low planting density of Bermuda grass respectively. The result of the statistical test by linear mixed model is also shown. “Interaction” means planting density  $\times$  treatment intensity. ns:  $p > 0.05$ , \*\*\*:  $p < 0.001$ .



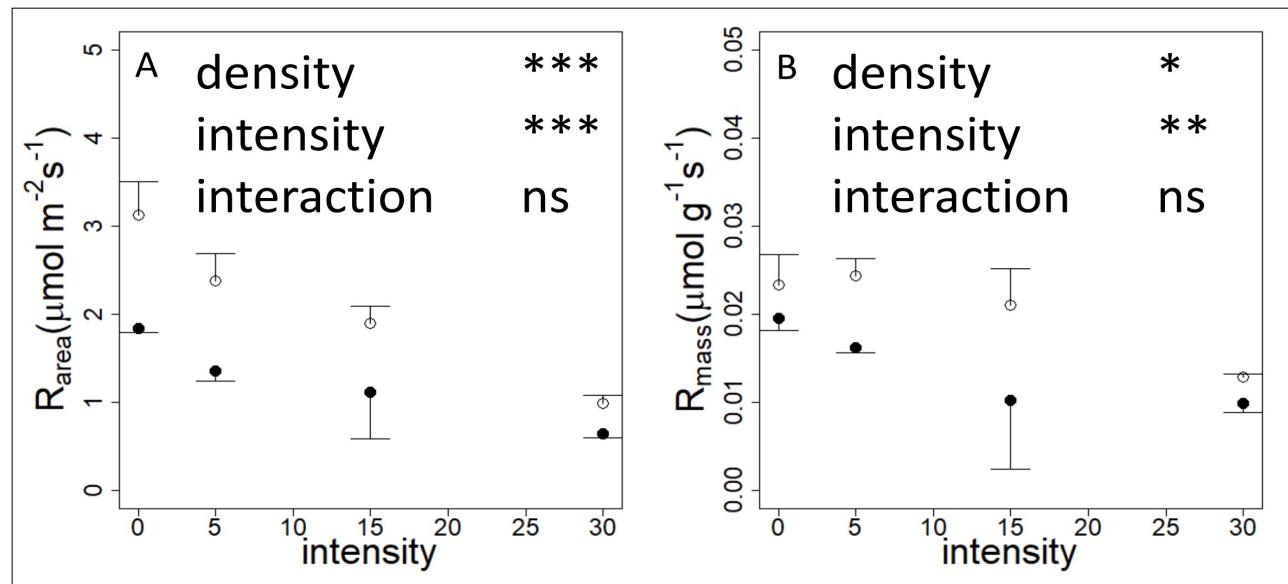
**Fig. 4.** Relationships between dead leaf mass and explanatory variables (planting density and compaction intensity) after the eighth traffic treatment. Opened circles and closed circles denote the high and low planting density of Bermuda grass respectively. The result of the statistical test by linear mixed model is also shown. “Interaction” means planting density  $\times$  treatment intensity. ns:  $p > 0.05$ , \*:  $p < 0.05$ , \*\*:  $p < 0.001$ .



**Fig. 5.** Relationships between root mass and explanatory variables (planting density and compaction intensity) after the eighth traffic treatment. Opened circles and closed circles denote the high and low planting density of Bermuda grass respectively. The result of the statistical test by linear mixed model is also shown. “Interaction” means planting density  $\times$  treatment intensity. ns:  $p > 0.05$ , \*\*:  $p < 0.01$ .



**Fig. 6.** Relationships between maximum photosynthetic rates (A: photosynthetic rates per ground area ( $A_{\text{area}}$ ), B: photosynthetic rates per leaf dry mass ( $A_{\text{mass}}$ ))) and explanatory variables (planting density and compaction intensity) after the eighth traffic treatment. Opened circles and closed circles denote the high and low planting density of Bermuda grass respectively. The result of the statistical test by linear mixed model is also shown. “Interaction” means planting density  $\times$  treatment intensity. ns:  $p > 0.05$ , \*\*\*:  $p < 0.001$ .



**Fig. 7.** Relationships between respiration rates (A: respiration rates per ground area ( $R_{\text{area}}$ ), B: respiration rates per plant dry mass ( $R_{\text{mass}}$ ))) and explanatory variables (planting density and compaction intensity) after the eighth traffic treatment. Opened circles and closed circles denote the high and low planting density of Bermuda grass respectively. The result of the statistical test by linear mixed model is also shown. “Interaction” means planting density  $\times$  treatment intensity. ns:  $p > 0.05$ , \*:  $p < 0.05$ , \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ .

## Discussion

### Soil properties

Traffic stress caused the compaction of the soil and hardened the soil structure. It was found that an increase in compaction frequency resulted in a notable hardening of the soil, and that repetitive traffic stress had a discernible impact on soil physical properties. Soil compaction presses the soil particles and reduces the space between them. This process leads to a decrease in the pore size of soil, which in turn results in the reduction of soil water permeability and retention of moisture (17, 18). In addition, a previous study concluded that a reduction in soil pores induced by soil compaction inhibits the gas exchange in the roots of turfgrass in a golf field in Poland (19). Hardened soil due to soil compaction can inhibit water uptake by roots (20).

Therefore, traffic stress can affect the physiological properties of plants related to water use and gas exchange through changes in soil permeability.

Growing roots push soil particles aside, and the size of soil pores decreases in the soil with large amounts of roots in grasslands (21). Root growth changes soil structure and this may affect gas permeability and moisture retention. Larger root mass in high-density plots may result in lower hydraulic conductivity in soil than in low-density plots, especially under low intensities of traffic stress. In addition, aboveground vegetation may also act as a buffer, which may mitigate the mechanical damage of traffic stress on the soil. Therefore, repeated soil compaction could change the physical properties of soil and affect water absorption for plants.

## Leaf and root mass

We found that traffic stress has a negative effect on turfgrass biomass, leading to a decrease in live leaves and an increase in dead leaves. This is consistent with previous studies that also found a decrease in turfgrass biomass under wear stress caused by traffic (5, 22). Leaf wear due to traffic stress results in reduced water content in the leaves, making them more susceptible to wilting (5). Additionally, an increase in leaf electrolyte leakage following compaction treatments was found, indicating the breakage of the cell membranes (4). Increased electrolyte leakage is associated with cell death, and damage to cells due to traffic stress may contribute to the higher rate of leaf mortality.

Leaf wear is a direct result of traffic stress, unlike underground parts, aboveground part biomass of turfgrass is more significantly affected by even a small degree of compaction stress (23). Despite the differences in the number of seeds planted, the high-density plot subjected to traffic stress did not maintain more leaves. Competition within the population in the high-density plot decreases the growth and maintains the constant biomass (24). Both competition within a population and wear can result in similar live leaf mass regardless of planting density, particularly in areas of high traffic intensity.

It is reported that soil compaction can lead to shorter root length (25, 26). Decreases in soil pore sizes due to compaction limit the gas exchange inside the soil, and this suppresses the development of roots (6, 18). In non-anaerobiosis conditions, soil compaction can limit the root elongation and decrease the diameter of xylem vessels in maize (27). These morphological and anatomical changes in roots are closely linked to water absorption capabilities. In contrast to the above-ground parts, which compete for light, the competitive effect of planting density is not significant in below-ground parts. Therefore, the root mass is also strongly influenced by planting density. Some studies have indicated that the manually reduced aboveground biomass resulting from mowing activities can have strong effects on soil compaction and reduce root biomass (28, 29). Consequently, the aboveground part of grasses can play a role in mitigating the effects of soil compaction caused by traffic stress.

## Photosynthesis and respiration per dry mass

The effects of traffic stresses on photosynthetic rates have not been widely studied, but some indirect measurements related to photosynthetic rates have been conducted. For example, some studies have suggested that the cellulose content in the cell wall indicates tolerance to wear on leaves, and the content of non-structural carbohydrates produced by photosynthesis reflects physiological activity (22). A previous study demonstrated physiological changes in turfgrass caused by the traffic stress using hyperspectral reflectance (23). They found that a decrease in chlorophyll content detected by hyperspectral reflectance after the traffic stress suggests a decrease in photosynthetic rates. Our study, which involved direct measurements of photosynthetic rates, supported these findings, showing similar results in low-density plots. It is important to note

that stomatal conductance drives photosynthetic rates (30), and both leaf wear and suppression of root development due to soil compaction may be related to photosynthetic rates. There were no decreases in photosynthetic rates in high-density plots despite the intensities of traffic stresses. Bermudagrasses with higher planting density may have high leaf cell rigidity, and this may result in less wear stress from the traffic treatments (31). Furthermore, individual leaf size in lower-density plots is expected to be higher than in high-density plots due to similar leaf mass regardless of planting density. This implies that larger individual leaves may experience more friction with soil particles, leading to more wear damage on low-density plots.

Soil compaction can cause damage to the roots, leading to a decrease in respiration rates per mass. The pressure from compaction can change the morphology and anatomy of the roots, causing them to become shorter and the cortex thicker (32). These morphological and anatomical changes in roots are important for maintaining the cell turgor pressure of fine roots, which helps them tolerate the friction between the soil and the roots (33). The loss of cell turgor pressure of roots by soil compaction restricts root growth and leads to a decrease in physiological activity (34).

## Photosynthesis and respiration per ground area

Photosynthesis and respiration rates per ground area include the effect of biomass (Fig. 3, 5) and physiological changes (Fig. 6B, 7B). Respiration rates per ground area decreased with the intensity of traffic stress due to the mixed effects of the decreases in biomass reduction and physiological reduction. Reductions in root length have often been shown to affect leaf quality (23, 35). Decreases in root activity can affect leaf activity because photosynthetic decline and leaf wilt occur in the absence of water uptake (36). The photosynthetic and respiratory processes were less active under the higher traffic stresses, and this means that both aboveground and belowground parts suffered from traffic damage.

The decrease in photosynthesis in response to traffic stress is more severe than the decrease in respiration, suggesting that traffic stress reduces the carbon budget of vegetation and that it is possible to have a negative budget under severe stress. A previous study showed that leaf wear and soil compaction cause a decline in leaf quality, which can be associated with pigment content, photochemical activity, and water content through non-destructive spectroscopic observations (37). Continuous monitoring of leaf quality using spectroscopic methods could indicate the simultaneous occurrence of morphological injury and physiological reduction on the surface of turfgrass (23).

It is to be noted that photosynthesis depends on changes in the light environment and  $A_{area}$ , but the difference between  $A_{area}$  and  $R_{area}$  can be used as a rough indicator of carbon budget. This study suggests that low photosynthetic carbon accumulation due to leaf wear and soil compaction have a risk for a negative carbon balance in low-density and high traffic stress plots. In such cases, further degradation may occur because less carbon is available for growth due to the negative carbon balance. In

high-density plots, there is less risk of degradation because they can utilize carbon after the leaf wear due to the positive carbon balance in this study. An earlier study demonstrated that high plant density can better tolerate traffic stress than low plant density in bermudagrass (38). Turfgrasses are planted in many areas, such as on slopes and sports fields, where they are exposed to trampling pressure and, therefore, need to be managed in a way that takes into account their resistance to physical stress (39). To manage turfgrass under trampling pressure, we need to ensure a positive carbon balance to offset the decline in photosynthetic rates due to leaf wear. Monitoring the carbon balance of turfgrass may be a valid method for assessing plant health and managing turfgrass fields.

## Conclusion

This study found that leaf mass, root mass, photosynthetic rate and respiration rate all decreased with the intensity of the traffic stress. The combination of traffic stress and planting density affects the carbon balance on the turfgrass field. Turfgrass under high-traffic stress and in low planting density is likely to decrease photosynthetic rates. It is important to maintain a positive carbon balance to keep the turfgrass field healthy under these conditions. For the management of turfgrass fields, it is important to maintain the positive carbon balance by reducing the intensity of traffic stress. If it is difficult to reduce the traffic intensity, higher planting density can moderate the damage to the soil by the traffic stress.

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## Authors' contributions

SO and KY took field measurements. SO made all analyses and KY wrote this manuscript. All the authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** The authors do not have any conflict of interests to declare.

**Ethical issues:** None

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