Biomechanical properties of growing and decaying roots of *Cynodon dactylon*

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Abstract

Aim

We measured the effects of growth and decay on the tensile strength of roots of *Cynodon dactylon*, considering different mortality causes common to agricultural land conversion (i.e., burning and herbicide application). Drivers of root strength changes have also been studies, including root chemical composition (i.e., cellulose and lignin).

Method

We applied three treatments to *C. dactylon* grass: (i) growth duration (60, 120 and 180 days), (ii) decay duration after burning (30, 60, 120, 180 and 360 days) and (iii) decay duration after herbicide application (15, 30 and 60 days). After each treatment, diameter, tensile strength, cellulose content and lignin content from root samples were measured (n = 303).

Results

Irrespective to the treatments, strength-diameter relations followed a negative power law ($R^2 > 0.6$). Increase in the median strength values due to grass growth was consistent with the increases in both the cellulose and lignin contents. Root decay caused by herbicide applications caused significantly greater and faster reduction in strengths than by burning treatment, because of the faster reduction of both the cellulose and lignin contents.

Conclusion

Root decay due to different causes of plant mortality can increase the susceptibility to erosion and slope instability during conversion of agricultural land. Measures on slope safety and erosion are vital when applying herbicides for weed clearance in farmlands because of faster deterioration of root chemical composition and root strength (compared to burning).

1. Introduction

In developing countries, the increasing demand for food, biofuels and market-driven agricultural products (i.e., agribusiness) are leading to agricultural land conversion on marginal lands, which are often characterised by less-fertile soils, invasive weeds and slopes susceptible to erosion and instability (Alexandratos and Bruinsma 2012; Fargione et al. 2008). Vegetation plays an important role to slope stability under intense rainfall (Stokes et al. 2014; Eab et al. 2015; Leung et al. 2017; Kamchoom and Leung 2018a; Nguyen et al. 2019; 2020), but land conversion means a change in vegetation cover (e.g., natural vegetation to farmland) and this can affect the slope safety (Pisano et al. 2017; Kamchoom and Leung 2018b; Kamchoom and Jotisankasa 2019; Leknoi and Likitlersuang 2020). For example, the deforestation of New Zealand's native forest by European colonisation determined a historically remarkable increase in landslides compared with the pre-European arrival period (Glade 2003). Moreover,

wildfires that are becoming more frequent and severe in many parts of the globe due to anthropogenic climate change also resulted in severe destruction of natural vegetation cover (Turco et al. 2018). Different weed clearance techniques have been adopted in agricultural practice, such as mechanical weeding (e.g., tillage), burning (i.e., combustion of the above-ground biomass) and application of herbicides (Ludwig 1986; Parish 1990; Clements et al. 2017). Land conversion to farmland procedures as well as wildfires can introduce root mortality and decay on large areas, consequentially affecting the root biomechanical properties and the abilities of root reinforcement to shallow soil stability (Watson et al. 1997; Vergani et al. 2017). Indeed, upon soil sliding, plant roots permeated into the soil matrix would mobilise their biomechanical properties (tensile or/and flexural) and provide mechanical reinforcement to resist the shear stress exerted to the soil (Stokes et al. 2014; Karimzadeh et al. 2021).

There exists a large volume of research in the soil eco-engineering literature with regard to the study of root tensile properties under different test conditions (e.g., Mao et al. 2012, Wu et al. 2021). Test results reported by Genet et al. (2005) and Zhang et al. (2014) highlighted the central roles of cellulose (polymer chains of glucose linked by hydrogen bonds) and lignin (complex cross-linked phenolic polymers with structural function) on the root tensile strength of *Pinus pinaster, Castanea sativa* and *Pinus tabulaeformis*. As roots age, these structural components are deposited within the cell walls (Campbell and Sederoff 1996). Loades et al. (2015) further attributed the increase in the root tensile strength of *Hordeum vulgare* (barley) with root aging (i.e., distance from root tip) to the increases in the cellulose and lignin contents during tissue development. While the mechanical effects of cellulose and lignin have been tested for different woody species, there is a lack of data on herbaceous species and in particular on fast-growing weeds widespread on marginal and abandoned lands.

Studying changes in root tensile properties and hence root reinforcement to soil due to root mortality and decay upon natural disturbances (e.g., storms and diseases) and human activities (e.g., harvesting) has also been a research focus since the 1960s (e.g., O'loughlin and Ziemer 1982; Watson et al. 1997, 1999; Vergani et al. 2014, 2016; Liu et al. 2017; Kong et al. 2018; Zhu et al. 2019; Chen et al. 2021). Despite receiving early interest in the literature, only a few studies are available. Existing work mostly focused on trees and woody species after woodland clearance or wildfires. While most of these existing studies show a reduction of tensile strength as the roots decayed, the time required to reach half of the original strength (t_{50}) varied from species to species. As summarised by Zhu et al. (2019), the t_{50} of woody species was normally in the order of year and can be up to 8 years (for *Picea abies*; Ammann et al. 2009). It is a general observation from the literature that decay did not introduce significant changes in the shape of the power law relationship between tensile strength and root diameter and caused mainly a downward shift of the curve. A recent study by Zhu et al. (2019) shows that cellulose and lignin contents are the primary components that explain the changes in the tensile strength.

Although agriculture land conversion has been one of the main human activities that cause soil erosion and instability (Glade 2003; Pisano et al, 2017), no or rare studies focus on the root decay of weeds or herbaceous species after clearance of land. Moreover, effects of different techniques to clear land and convert it to farmland (i.e., means to introduce root mortality or decay) such as burning and herbicide

applications to root biomechanical property changes have never been investigated. Whether different techniques of land clearance and conversion (e.g. burning or herbicide application) would translate in any differences of the root tensile properties due to decay, or not, is unknown. These are important, yet unanswered, research questions that have direct implication to mitigate soil erosion and instability caused by land conversion.

The objective of this study is to investigate how the growth and decay of fibrous roots of a grass species *Cynodon dactylon* would affect the tensile properties that have important implication to the shallow slope stability and erosion control. The following hypotheses are made:

- Older *C. dactylon* have higher tensile strength than the younger ones.
- Decay of *C. dactylon* roots causes a reduction of root tensile strength.
- Different techniques of farmland clearance (e.g., grass burning versus herbicide application) introduce different effects on the changes in root tensile strength (e.g., different root decay after plant killing).
- Changes in root chemical compositions such as cellulose content and lignin contents due to root growth and root decay can explain the changes in root tensile strength.

2. Materials And Methods

2.1 Root and soil materials

Roots of *Cynodon dactylon* (common name: bermuda grass) were tested. It is a warm-seasoned perennial grass species (Skerman and Riveros, 1990) and spreads mainly by rhizomes (underground stems) and stolons (horizontal aboveground stems). *C. dactylon* has been classified as one of the most important weeds on global scale (Holm et al, 1977). *C. dactylon* can rapidly invade agricultural landscape and cause serious yield losses. It is extremely difficult to eradicate. *C. dactylon* has been included in the Global Invasive Species Database (GISD 2010). This grass has dense fibrous root system in shallow soil and has thus been commonly used for soil erosion control (Faucette et al., 2006; Ng et al., 2014). *C. dactylon* was sown in vermicompost which was rich in nutrient to facilitate root growth for a month. Clayey sand (following the unified soil classification system; ASTM D2487) was used to represent the soil found in many parts of Thailand, especially in natural and man-made slopes. The soil was slightly acidic, with a pH value of approximately 5. The clayey sand was compacted to a dry density of 1520 kg/m^3 in a cylinder (90 mm-diameter, 115 mm-depth). The young grass, together with the vermicompost at 15 mm-depth, was overlain on the compacted clayey sand. In total, 30 cylinders were prepared. All cylinders were then placed in a glasshouse.

2.2 Growth and decay treatments

Three treatments were applied to the 30 cylinders, one for root growing (9 cylinders) and two for root decay (21 cylinders). All the cylinders were left in the glasshouse for root growth for 60, 120 and 180 days

from Aug 2018 to Jan 2019 (denoted as G60, G120 and G180), which was the wet season in Thailand. During this period, the average daily temperature was 33°C and the glasshouse was open for air ventilation. All cylinders received about a continuous 10 h of sunlight daily and were irrigated with tap water twice a week.

For the cylinders that were used to study root decay, two treatments were applied, namely burning and herbicide application. For the burning treatment (12 cylinders), all the above-ground grass biomass was ignited until the shoot and leaves were completely burnt. The duration of burning varied from one to six hours, depending on the amount of the biomass. The soil surface was exposed to fire, like what would happen in the field. Fire retardant foam (polyisocyanurate foam) was applied to the top part of container so they were prevented from burning. After burning, the cylinders were left for root decay in the soil for 30, 60, 120, 180 and 360 days (denoted as B30, B60, B120, B180 and B360). For the herbicide treatment (9 cylinders), Propanil (N-(3,4-Dichlorophenyl) propanamide) at 36% W/V in water solution in two does was applied to the columns together with irrigation. Propanil is a widely used herbicide for land clearing and weed control in Thailand agricultural practice. The 9 cylinders for these treatments were left for root decay for relatively shorter duration (i.e., 15, 30 and 60 days; denoted as H15, H30 and H60). All the cylinders subjected to root decay (burning and herbicide) were irrigated twice a week during the entire decay period to avoid excessive soil drying.

After applying each treatment, roots from the clayey sand (i.e., not from the vermicompost) were collected for measuring the tensile strength and subsequently the cellulose and lignin contents. The roots were separated from the soil by wet-sieving, and the bare roots were submerged in distilled water for more than 12 h before testing.

2.3 Measurement of root tensile strength

The hydrated roots were trimmed to 50 – 70 mm long segments. Only segments that were free of tortuosity or tissue damages were selected for testing. The average root diameter was determined by averaging the diameters measured at three positions of each segment (near the two tips and at the mid-length) by an electronic slide gauge. Root diameters ranged between 0.1 – 1.9 mm were randomly selected for the tests, covering the most possible range of *C. dactylon*.

The root segments were subsequently subjected to a uniaxial tensile test, at a constant extension rate of 0.1 mm/s, by using a universal testing frame (TTR MUL-125). To ensure an intimate contact between the root segments and the grips and to minimise slippage, a couple of thin wooden pieces were used to tighten the root segments (Nilaweera and Nutalaya, 1999). The gauge length of each root segment was 50 – 60 mm. Different capacities of load cell (20 N, 50 N and 500 N) were used to measure the force required to tension the roots, depending on the diameter of the root segments tested. Samples that were failed at or near the clamps' edges were discarded. The average test duration was approximately 5 minutes. After testing, each root segments were weighed again and then oven-dried at 60 ^{o}C for 24 h to

determine the initial and final root water content (i.e., before and after tensioning). The average loss of root water during the tests was less than 5% of the hydrated root water content.

Based on the test results, tensile strength (T_r), which is defined as the maximum stress required to break a root segment, was calculated by Eq. (1),

$$T_r = \frac{F}{\pi\left(\frac{d}{2}\right)^2} \quad (1)$$

where F is the force at root breakage; and d is the average root diameter before tensioning.

2.4 Measurement of cellulose and lignin contents

The method used to measure the cellulose and lignin content followed Leavitt and Danzer (1993). This method, in principle, first removed organic compound and then lignin polymers from the root material. Thus, the remaining content can be regarded as cellulose compound.

After oven-drying at 60 ^{o}C for 24 h, the root samples were ground into fine powder. The dry root powder was then weighed using a high-precision electronic balance accurate to 0.001 mg. The first step was to remove the organic compound. Each root sample was placed in an extraction chamber of Soxhlet apparatus equipped with a flask containing a 700 mL solution of toluene 99% and ethanol 96% (2–1; v/v). The mixture was heated until boiling for 12 h. After extraction, the toluene ethanol was replaced with 700 mL of ethanol and heated to the same temperature for 12 h. Then, the mixture was removed from the Soxhlet apparatus and submersed in boiled distilled water for 6 h. The mixture was then rinsed with distilled water, oven-dried at 60 ^{o}C for 24 h. Finally, the remaining lignin and cellulose contents were weighed.

The second step was to remove lignin content. The mixture was placed in a beaker containing 700 mL of distilled water, 7.0 g of sodium chlorite ($NaClO_2$), and 1.0 mL of acetic acid ($C_2H_4O_2$). The mixture was shaken by a magnetic agitator and heated to 70 ^{o}C for 18 h. The mixture was then rinsed with distilled water, oven-dried at 60 ^{o}C for 24 h and weighed. The final weight was the weight of the cellulose. The lignin weight was determined by the difference in the final weight and the weight after the first step. The cellulose and lignin contents were determined by the ratio over the initial weight of the root samples (in g/g).

2.5 Statistical analysis

Statistical analysis was performed using GenStat 17th Edition (VSN International) and SigmaPlot13 (Systat Software Inc). Significant differences were assessed with one-way analysis of variance (ANOVA), followed by post hoc Tukey's test. Data that did not follow a normal distribution were log- or square-root

transformed before conducting the ANOVA. Results were considered statistically significant when p-value ≤ 0.05. The variability of averaged data is expressed as ± standard error of mean (SEM).

3. Results

3.1 Effect of grass age

Within the growth period of 180 days, the average root diameter and tensile strength is 2.14 ± 0.10 mm and 26.05 ± 1.71 MPa, respectively (Table 1). Although the average root diameter highlighted a slight increase with grass age (Fig. 1a), both the average diameter and tensile strength did not show significant difference during the entire growth period (i.e., 60, 120 and 180 days; Fig. 1a b). For any given growth period, the relations between tensile strength and root diameter followed a negative power law ($R^2 > 0.60$; Fig. 2 and Table 1).

The cellulose and lignin contents of *C. dactylon* ranged between 17.5% – 70.6% and 1.0% – 37.2%, respectively. Interestingly, unlike the root diameter and root strength, the growth period highlighted significant effects on the cellulose and lignin contents between 60 and 120 days of grass growth (p-value < 0.001; Figs. 3a b). However, no statistical difference was observed after 120 days. Figure 4 shows that the cellulose content has a negative relation with root diameter (Fig. 4a), and the diameter-dependency is affected by the growth period (Table 2). On the contrary, the lignin content has no, or weak, dependency on root diameter (Fig. 4b).

3.2 Effects of grass burning and herbicide application

Root decay, induced by burning or herbicide, did not cause significant change in the root diameter; Fig. 5a, b). For the given decay durations of 30 and 60 days, the root diameter has no significant difference between the two decay treatments (i.e., burning vs herbicide; Fig. 5c).

The root tensile strength, on the contrary, was affected by the two decay treatments (Fig. 6), and the average value was reduced with an increasing decay duration in both cases. After an initial increase in average tensile strength (+ 46% after 30 days; not significant), the average strength dropped by 30% from 24.64 ± 1.99 MPa to 17.27 ± 1.83 MPa after 360 days after grass burning (Fig. 6a). At a given decay duration (e.g., 30 days), the herbicide application introduced a significantly greater reduction in strength than the burning treatment (p-value < 0.001; log-transformed data); Fig. 6c). Irrespective to the decay treatment, the strength displayed negative power law relations with the root diameter (Fig. 7 and Table 1). By comparing the exponents in the equation, root decay did not introduce a major change to the relation between diameter and strength (i.e., no change in the shape of the power law relation), but to shift the curve down in the strength-diameter space as the strength dropped in all diameter classes.

The root cellulose content was also significantly affected by the decay treatments (Fig. 8). For the burning and herbicide treatments, the cellulose content reduced by 63% (18.58 ± 1.64%, 360 days after burning) and 58% (20.59 ± 1.80, 60 days after herbicide application), respectively. The rate of the

cellulose content reduction was less significant at 180 days after burning (Fig. 8a) and at 30 days after herbicide application (Fig. 8b). When comparing the two decay treatments (Fig. 8c), the rate of cellulose content reduction induced by the herbicide application was much faster than that by burning for the same period of root decay (e.g., H60 roots were 85% weaker than B60; Fig. 8c). The cellulose content was negatively correlated with root diameter (Fig. 9), and the diameter-dependency was similar between the two treatments, irrespective to the duration of root decay considered (compared the fitting coefficients in Table 2).

The lignin content highlighted a significant drop from 21.96 ± 1.42% (before burning; 180 days old grass) to 13.85 ± 1.78% at 360 days after burning (= 43% decrease; Fig. 10a). This shares a similar trend as the corresponding strength data presented in Fig. 6a. For the herbicide treatment (Fig. 10b), the lignin content was unchanged during the first 30 days of herbicide application, but it then displayed a significant drop of 46% after 60 days (compared to the control value). As shown in Fig. 10c, the herbicide treatment influenced the lignin content (so as the tensile strength and cellulose content) earlier than the burning treatment. The lignin content showed no significant relation with root diameter (Fig. 11).

4. Discussion

Our study highlighted a fast decay of strength of fibrous roots following grass death (e.g., 30 days). This is the first study to highlight the significant effects of different land clearing methods (i.e., burning vs herbicide) on the grass-root decay and strength loss. The herbicide application introduced a greater reduction of root strength than burning for a given decay duration.

4.1 Tensile strength of C. dactylon roots

Root tensile strength values measured in *C. dactylon* (Table 1) fell in the strength range recorded for fibrous roots of grasses (i.e., non-woody roots; Comino et al. 2010; Loades et al. 2013; Boldrin et al. 2021, Wu et al. 2021). However, the maximum strength values largely differed for fibrous roots of different species. For instance, while Boldrin et al. (2021) reported a maximum strength value smaller than 40 MPa in three grasses common in European pastures, Comino et al. (2010) reported strength values up to 365 MPa for similar species. However, it should be noted that the range of tested diameters of different grasses reported in the literature varied largely. When the same diameter range was considered (e.g., 0.1–1.9 mm), the roots tested in the present study generally has similar maximum strength values to those reported by Boldrin et al (2021). Furthermore, the strength-diameter relation of *C. dactylon* followed the negative power law model commonly found in the literature on fibrous roots (Mao et al. 2012). This relation was observed irrespective of the decay treatment applied to the grass in the present study (i.e., grass age or decay; Table 1; Figs. 2 and 7).

The negative strength-diameter relations found in the literature has been explained with the chemical composition of root tissues in different diameter classes (Genet et al. 2005; Zhang et al. 2014). However, these previous explanations were based on woody species and often contradictory. While Genet et al.

(2005) found positive relations between cellulose and tensile strength in *P. pinaster* and *C. sativa* roots, Zhang et al. (2014), who tested *P. tabulaeformis*, observed the opposite trend (i.e., negative relations). Zhang et al. (2014) explained the negative relation between diameter and strength with a decrease in the lignin-cellulose ratio with root thickening. Our results for fibrous roots showed that both the tensile strength and cellulose content decreased consistently with an increase in root diameter (Figs. 4a and 9), in agreement with Genet et al. (2005). In contrast, the lignin content showed no, or weak, relation with the root diameter (Figs. 4b and 11). It should be noted that grass roots have no secondary xylem and hence no lignin-rich tissues such as woody roots of trees (Cutler et al. 2009, Roumet et al. 2016). Although the lignin content did not highlight a clear relation with diameter in our study, its contribution to plant biomechanics has been well recognized (Niklas et al. 1992).

The large tensile strength and dense root mat of *C. dactylon* make this species an ideal "engineer plant" for soil bioengineering uses. For instance, the strength values are similar to those of *Chrysopogon zizanioides* L. (vetiver grass; Mickovski and van Beek, 2009; Mahannopkul and Jotisankasa 2019; Wu et al. 2021; Karimzadeh et al. 2021), which has received wide popularity for erosion control and slope stabilisation in tropical/subtropical regions (National Research Council 1993). However, *C. dactylon* may be more suitable for erosion control, given their shallow root system (< 0.5 m; Ng et al. (2013)), in contrast to the deep-rooted *C. zizanioides*, whose roots can penetrate down to 3–5 m depth (Hellin and Haigh 2002). *C. dactylon* has a great potential for fast cover of soil exposed to surface erosion by runoff water, given its pioneering ability and fast diffusion by long rhizomatous stolons (Norris et al. 2008).

Grass age (e.g., 60 to 180 days) exhibited only a small influence on the root tensile strength. Although the median values of strength increased with grass age (i.e., 17.5 MPa after 60 days; 22.3 MPa after 120 days; 22.7 MPa after 180 days), no significant differences were found between root population samples from grass of different ages. The tendency of root strengthening with plant aging may be explained by the larger amount of older roots, which have more cellulose and lignin contents. Indeed, previous studies on fibrous roots have found a notable increase in strength along a root axis as a consequence of root aging (Dumlao et al. 2015, Loades et al. 2015, Boldrin et al. 2021). These studies suggested that the increase in root strength with aging is the result of cellulose and lignin deposition. In the present study, the observed significant increases in the cellulose and lignin contents with grass age (Fig. 3) is consistent with the change in the median value of tensile strength, and they are also in agreement with previous hypotheses in the literature on root biomechanics (Dumlao et al. 2015, Loades et al. 2015, Boldrin et al. 2021). However, it should be noted that roots samples from the same plant might be largely different in age (i.e., both old and young root sections).

4.2 Effects of root decay

Root decay following the herbicide application and burning translated in a significant reduction of root tensile strength (Figs. 6, 7). For instance, a 40% decrease in strength was recorded after only 60 days from the herbicide application (i.e., from 24.64 ± 1.99 to 14.87 ± 1.32 MPa). On the other hand, the strength reduction following burning was slower compared to the herbicide treatment. For example, a 30% drop in strength was observed 360 days after burning. The observed strength reductions were faster

than those reported in the literature, where a 50% strength drop generally required one to eight years, depending on the species and both the biotic and abiotic conditions during the process of decay (Watson et al. 1999, Zhu et al. 2020). The difference from the data in the literature can be explained by the fibrous (i.e., non-woody) nature of the roots tested in the present study. Indeed, existing studies on root decay and biomechanical properties focused on woody roots of trees, and no or little attention had been given to fibrous roots of grasses (e.g., Vergani et al. (2016) and Zhu et al. (2020) studied woody roots of trees). For instance, roots of *Picea abies* showed a 50% drop in strength only after eight years of decay (Ammann et al. 2009). The large differences in terms of biomechanical response to root decay between woody (literature) and fibrous (the present study) roots are the results of different root characteristics such as diameter, root anatomy and tissue composition. While the tested fibrous roots were thinner than 2 mm, the woody roots tested during decay in the literature ranged from less than 1 mm to more than 10 mm (e.g., Vergani et al., 2016, Watson et al. 1998, Zhu et al. 2020). Indeed, while woody roots of shrubs and trees exhibit secondary radial growth (i.e., root thickening) with ageing, fibrous roots of grasses have no secondary radial growth and hence less anatomical changes with ageing (Cutler et al. 2009, Roumet et al. 2016). In general, thinner roots are more prone to decay, given the larger surface area per volume exposed to detritivores and bacteria, promoting the decomposition of organic matter. However, it is noteworthy that physiology, lifespan and decay of individual roots are not simply functions of the root diameter but being influenced by soil nutrients, the degree of mycorrhizal infection and root topology (Pregitzer, 2002). Recent studies highlighted that chemical functional parameters (such as carbon and nitrogen concentrations) and respiration explained decomposability of fine roots (Prieto et al. 2016, Roumet et al. 2016). In general, herbaceous species that occupy frequently disturbed environments exhibit root traits associated with a fast resource acquisition strategy (high growth and respiration rate) and high decomposability. On the contrary, woody species exhibit a more resource-conservative strategy characterized by slow growth, thicker roots, greater longevity and lower decomposability (Roumet et al. 2016). Moreover, a higher decomposability was also observed in shallow roots and agroecosystems compared with deep roots and forests (Prieto et al, 2016).

In all decay treatments (Table 1), the tensile strength displayed negative power law relations with root diameter, which have been commonly reported for both fibrous and woody roots in the literature (Boldrin et al. 2021; Mao et al. 2012). Therefore, root decay did not introduce a major change in the shape of the strength-diameter curves. However, the root decay progressively shifted the curve down in the strength-diameter space as the strength reduced in all diameter classes (Fig. 7). A similar result was reported by Zhu et al. (2020) for woody roots of *Symplocos setchuensis* (e.g., see Fig. 7 in the present study and Fig. 1 in Zhu et al. (2020)).

Root decay and weakening (i.e., reduction of tensile strength) was significantly affected by the cause of root mortality (i.e., burning or herbicide treatment). Indeed, at a given decay duration (e.g., 30 days), the herbicide application introduced a greater decrease of strength than burning (Fig. 6c). To the best of our knowledge, our results are the first evidence of the effect of the mortality cause on root decay and strength reduction. Although previous studies (mainly on trees) tested root decay after different causes of root mortality, no systematic comparisons were made. Most of the studies on root decay focussed on

timber harvesting and no study considered different methods to clear lands (i.e., agricultural land conversion) from herbaceous species such as herbicide application and burning. Despite wildfires and land conversion have become global concerns within the climate warming scenario, with unprecedented effects and scale (Jolly et al. 2015), only limited studies investigated the effect of vegetation burning on root decay, changes in biomechanical properties and hence soil reinforcement (e.g., Vergani et al. 2017). Vergani et al. (2017) investigated the decrease in root strength and soil mechanical reinforcement following the burning of a *Pinus sylvestris* woodland. After 4 years, the roots tested by Vergani et al (2017) exhibited a notable strength reduction (e.g., 24% strength of a living root). In our study, fibrous roots exhibited on average a 30% reduction in strength in only 360 days from burning (Fig. 6).

Root strength after burning highlighted an initial increase (+ 47% increase after 30 days) followed by a root weakening (-52% at 360 days compared to the value at 60 days). This trend of root strength variation due to plant death was previously reported by Watson et al. (1998) in the roots of *Kunzea ericoides*. Indeed, the woody roots they tested highlighted an initial 27% strength gain followed by a constant strength reduction with decay duration. The strength increase observed in 60 days after *C. dactylon* burning can be explained by the loss of root moisture and consequent diameter shrinkage, in agreement with previous hypothesis formulated by Watson et al. (1998) for *K. ericoides* roots and recent studies by Boldrin et al. (2018) for *Ulex europaeus* roots and Wu et al. (2021) for *C. zizanioides* roots. Indeed, our results presented in Fig. 5 highlight a notable diameter reduction in 30 days after burning. Following the initial strength increase, root strength reduced to relatively stable values, recorded after 180 days (16.58 ± 1.58 MPa) and 360 days (17.27 ± 1.83 MPa). Therefore, it can be hypothesised that: firstly, the strength increases due to a calculation artefact induced by diameter decrease (Eq. 1); then, the decomposition process driven by soil biota weakens the root material; finally, the strength reaches stable values (e.g., 180 and 360 days) as highly decomposable tissues are destroyed and the remaining root tissues are more resistant to biotic attack (e.g., high carbon: nitrogen ratio) and hence longer processes are needed to fully degrade these recalcitrant tissues. Future work is needed to corroborate this hypothesis by testing for a much longer decay period.

On the other hand, herbicide application resulted in a faster weakening of roots (compared to the burning treatment). For instance, after 60 days of root decay, the strength for the case of herbicide application (14.87 ± 1.32 MPa) was 54% of that of the roots subjected to the burning treatment (27.64 ± 4.86 MPa; Fig. 6c). Unlike the burning treatment, the roots with the herbicide treatment showed no initial strength gain (Fig. 6a, b). Indeed, root diameter, which may have caused a strength gain following the burning treatment, did not change during the decay in the herbicide treatment (Fig. 5c). Hollis et al. (2019) found a reduction in mechanical resistance (force at failure) in the roots of *Spartina patens* (emergent macrophyte in wetlands) growing in soil contaminated with atrazine herbicide. However, no lethal dose or root decay in time was investigated in this study, which focussed on wetland pollution. To our best knowledge, our work is the first study to investigate the effect of herbicide application on root decay and tensile strength in comparison with both living roots and roots subjected to burning. The difference between the decay in the burning and herbicide treatments can be explained by different mechanisms leading to root mortality and decay. The effect of fire on roots varies, depending on root depth,

temperature and duration of fire (Swezy et al. 1991, Vergani et al. 2017). While in shallow soil, fire can directly induce root mortality due to high temperature, in deeper soil root mortality is the consequence of severe damage of the above-ground plant organs. In our experiments, both mechanisms may have contributed to root mortality and strength variability. Moreover, the high temperature during burning may have induced a moisture loss from soil and roots, with consequent diameter shrinkage and strength gain (Fig. 6a). On the other hand, roots in deeper soil may have died slowly as they may be unable to maintain root growth and metabolism due to the lack of photosynthate supplied by above ground organs (completely burnt). Moreover, burning may have affected soil biota responsible for the degradation of organic matter (e.g., root) due to high temperatures and potential toxic pyrogenic compounds (Certini et al. 2021). In contrast, herbicide application may have induced a faster and more systemic death of all plant organs, without any physical effect on soil and root tissues (e.g., drying in burning treatment). Vergani et al. (2017) suggested a faster root weakening after woodland burning, compared to post timber-harvesting. However, different species and study sites considered among different studies did not allow for a fair comparison and robust conclusion on the faster strength reduction after fire. Understanding and predicting root weakening due to decay are fundamental to assess slope susceptibility to erosion and shallow landslides. For instance, the period of highest susceptibility for shallow landslides varies between 3 to 20 years after timber harvesting in relation to species (woody roots) and environmental factors. Although in this condition a rainfall event is still required to trigger the slope failure (e.g., decrease in soil matric suction), a smaller threshold is needed (Sidle and Bogaard 2016). Our results on root weakening following plant burning or herbicide application (Fig. 6) can give insight on the erosion vulnerability after clearance of marginal lands, which are often characterised by invasive weeds and steep slopes prone to erosion and instability (Alexandratos and Bruinsma 2012; Fargione et al. 2008).

4.3 Root tissue composition behind strength reduction

The reduction of root tensile strength can be mechanistically explained by the loss of structural components such as cellulose and lignin. Indeed, these are the main chemical compounds contributing to plant biomechanical properties in both above- and below-ground organs (Genet et al. 2005; Niklas 1992; Zhang et al. 2014). The cellulose content showed a significant and constant reduction following grass death (Fig. 8a). After 60 days from grass death (burning and herbicide application), there were 17% and 58% drops in the cellulose content of the roots with the burning and herbicide treatments, respectively. Furthermore, the difference in the reduction of the cellulose content between the herbicide and burning treatments were consistent with those in strength reduction between the two treatments (Figs. 6c and 8c). Zhu et al. (2020) also explained the reduction of tensile strength in woody roots due to a reduction in the cellulose content. However, their reported reduction in the cellulose content was slower than what we found in our fibrous roots. For example, only 8% of the cellulose content was lost after a three-month decay in the woody roots of *Symplocos setchuensis* (Zhu et al. 2020).

In all the treatments, the cellulose content was negatively correlated with diameter (Fig. 9). Although the cellulose content varied between root diameter classes, the reduction in the cellulose content over time

was consistent among diameter classes (i.e., no effect on the cellulose content-diameter relations). The lignin content showed a smaller and slower reduction compared to the cellulose content. Indeed, notable drops of the lignin content were observed after 180 days of burning (-28%) and 60 days of herbicide application (-46%). Zhu et al. (2020) also recorded a reduction in the lignin content of 5% after three months and 8% after 12 months from tree felling. The slower reduction in the lignin content compared with the cellulose content can be explained by its recalcitrant nature. For instance, the slower reduction in the lignin content compared to the cellulose content has been observed for both root material (Zhu et al. 2020) and foliar litter (Yue et al. 2016). In fact, the lignin-nitrogen ratio is a recognised predictor of decomposability and soil carbon sequestration (Prescott 2010). In our study, the reduction of both the cellulose and lignin contents led to root weakening, but the reduction in the lignin content, hence its mechanical effect on strength, was smaller and observed in later stage of root decay.

Concluding Remarks

Our data highlights that irrespective to the treatments applied (growth duration, decay duration by burning or herbicides), all tensile strength-diameter of the roots of *Cynodon dactylon* tested (n = 303) followed a negative power law relation ($R^2 > 0.6$). Growth effects have minimal influences on root diameter during 180-day growth period, but they have significant effects on the increase in median strengths. The strength gain was consistent with the increases in both the cellulose and lignin contents. Root decay due to burning or herbicide application caused significant reductions in both the cellulose and lignin contents, accompanying by the drops of root strength (i.e., root weakening). The root decay effects did not change the shape but shifted the strength-diameter relations downwards. Compared to the burning treatments, herbicide application introduced greater and faster degradation of the cellulose and lignin contents, which explained the more significant and faster root weakening. Future research is needed to assess the biomechanical effect of grass-root decomposition during a longer time (> 360 days) and field conditions, as well as the effect of root decay on soil shear strength.

Declarations

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Author contribution

VK, AL and SL conceived the research idea, designed the experiments and produced the first draft of the paper. CS conducted the experiments. DB and AL contributed to writing the paper. VK and SL commented the paper.

Date availability statement

The data that supports the findings of this study are available from the first and corresponding authors upon reasonable request.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Tables

Table 1. Summary of the data of diameter (*d*) and tensile strength (T_r; mean ± SEM) of *C. dactylon*. Best-fit equation, *p*-values and R^2 are given for the strength-diameter relations.

Treatment	Range of *d*, mm	n	T_r, MPa	Fitting equation	*p*-value	R^2
Grass growth						
G60	0.1 – 1.9	30	24.54±3.84	$T_r = 16.85d^{-1.06}$	<0.001	0.64
G120	0.3 – 1.8	30	28.98±2.82	$T_r = 24.52d^{-0.88}$	<0.001	0.80
G180	0.3 – 1.8	30	24.64±1.99	$T_r = 23.66d^{-0.73}$	<0.001	0.77
Burning treatment						
B30	0.3 – 1.8	26	36.10±4.70	$T_r = 23.69d^{-0.97}$	<0.001	0.68
B60	0.3 – 1.9	30	27.64±4.86	$T_r = 14.91d^{-1.74}$	<0.001	0.82
B120	0.3 – 1.9	24	9.10±2.11	$T_r = 17.99d^{-0.81}$	<0.001	0.87
B180	0.3 – 1.9	25	16.58±1.58	$T_r = 14.55d^{-0.69}$	<0.001	0.62
B360	0.3 – 1.7	26	17.27±1.83	$T_r = 14.75d^{-0.69}$	<0.001	0.58
Herbicide treatment						
H15	0.3 – 1.9	30	22.00±2.03	$T_r = 17.78d^{-0.75}$	<0.001	0.80
H30	0.3 – 1.7	25	18.34±2.68	$T_r = 14.68d^{-0.97}$	<0.001	0.63
H60	0.3 – 1.9	27	14.87±1.32	$T_r = 12.89d^{-0.66}$	<0.001	0.71

Table 2. Summary of the data for cellulose content (CL) and its relationship with root diameter (*d*). Best-fit equation, *p*-values and R^2 are given for cellulose-diameter relationships.

Treatment	**Cellulose content [%]**	**Fitting equation**	***p*-value**	**R^2**
Grass growth				
G60	31.51 ± 1.59	$CL = -5.34d + 36.99$	0.097	0.09
G120	52.80 ± 2.14	$CL = -15.49d + 69.46$	<0.001	0.44
G180	49.57 ± 2.04	$CL = -11.69d + 63.01$	0.005	0.25
Burning treatment				
B30	48.06 ± 2.06	$CL = -11.99d + 58.71$	0.003	0.31
B60	41.21 ± 2.05	$CL = -11.83d + 53.59$	0.009	0.23
B120	31.20 ± 1.95	$CL = -11.78d + 45.14$	0.002	0.37
B180	24.07 ± 2.15	$CL = -14.22d + 38.40$	0.001	0.39
B360	18.58 ± 1.64	$CL = -8.97d + 27.27$	0.016	0.23
Herbicide treatment				
H15	29.41 ± 1.93	$CL = -9.82d + 39.32$	0.006	0.24
H30	19.76 ± 2.04	$CL = -13.31d + 33.29$	0.006	0.28
H60	20.59 ± 1.80	$CL = -8.70d + 29.48$	0.010	0.23

Figures

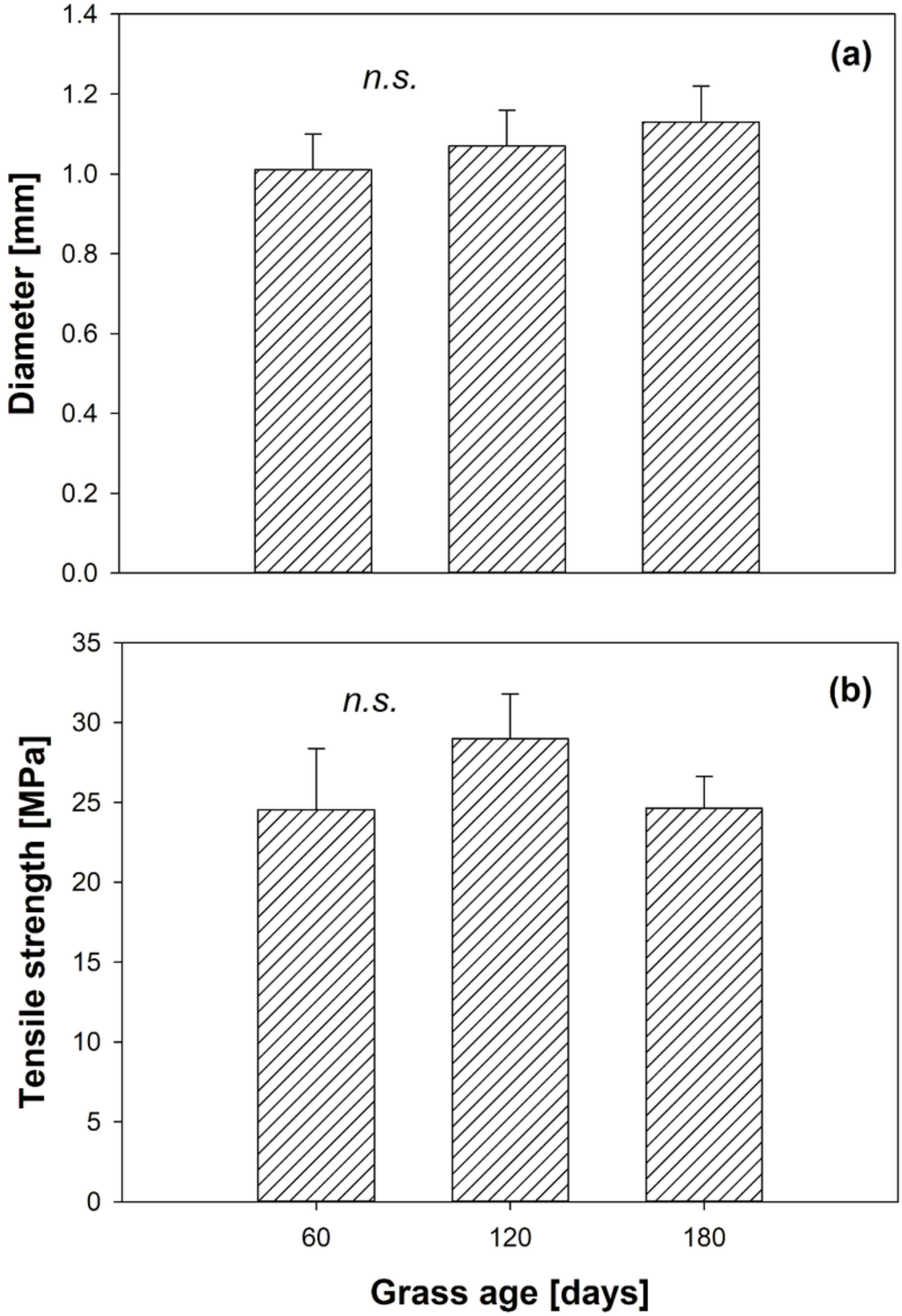

Figure 1

Mean (a) diameter (n = 30) and (b) tensile strength (n = 30) (± standard error of mean) of Cynodon dactylon roots randomly sampled from 60, 120 and 180-day-old grasses. n.s. indicates a non-statistically significant difference between treatments, as tested using one-way ANOVA.

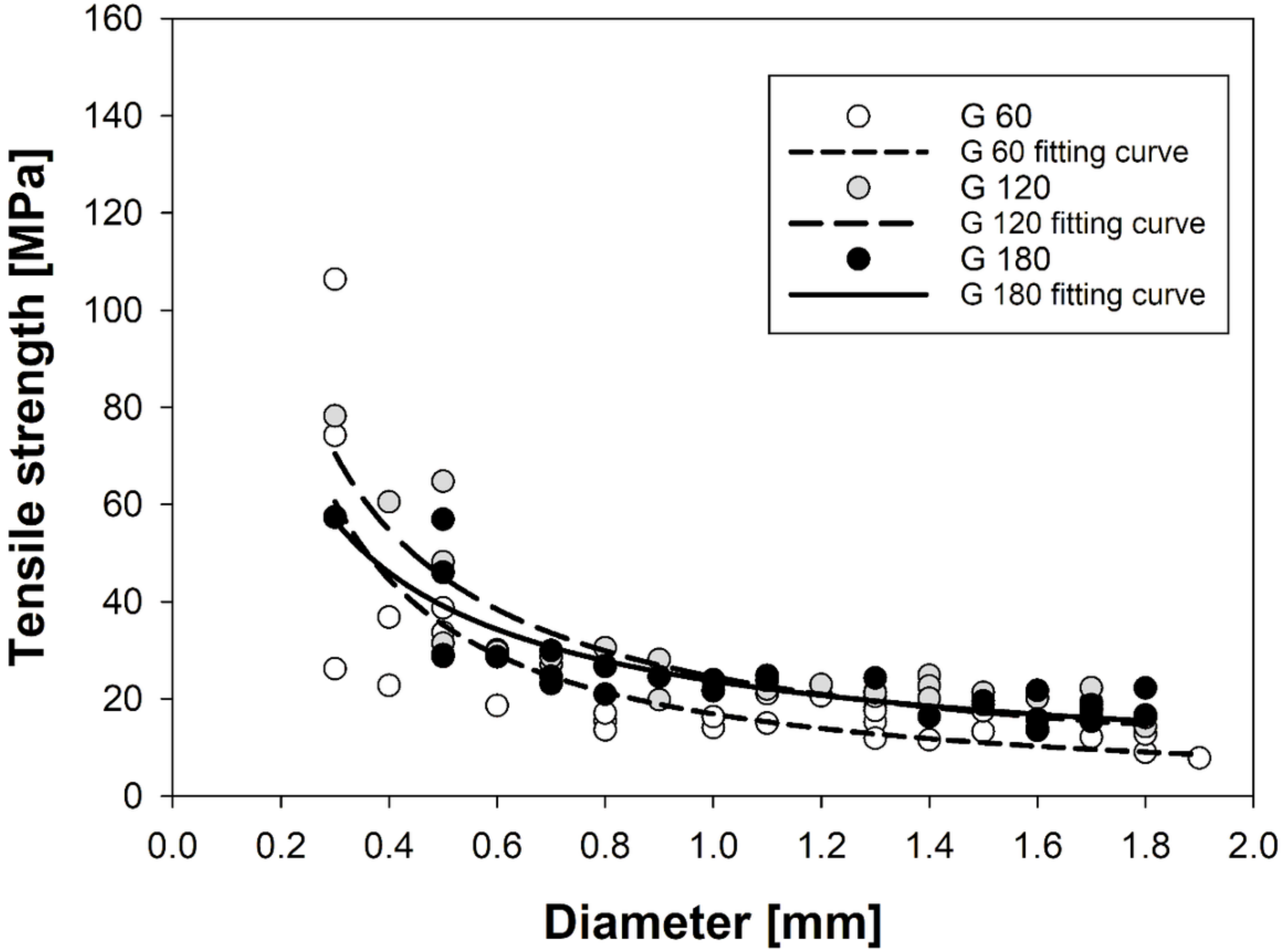

Figure 2

Relations between tensile strength and root diameter of C. dactylon roots randomly sampled from 60, 120 and 180-day-old grasses. Solid lines represent the best-fitted curve. The fitting equations and goodness-of-fit (R2) are given in Table 1.

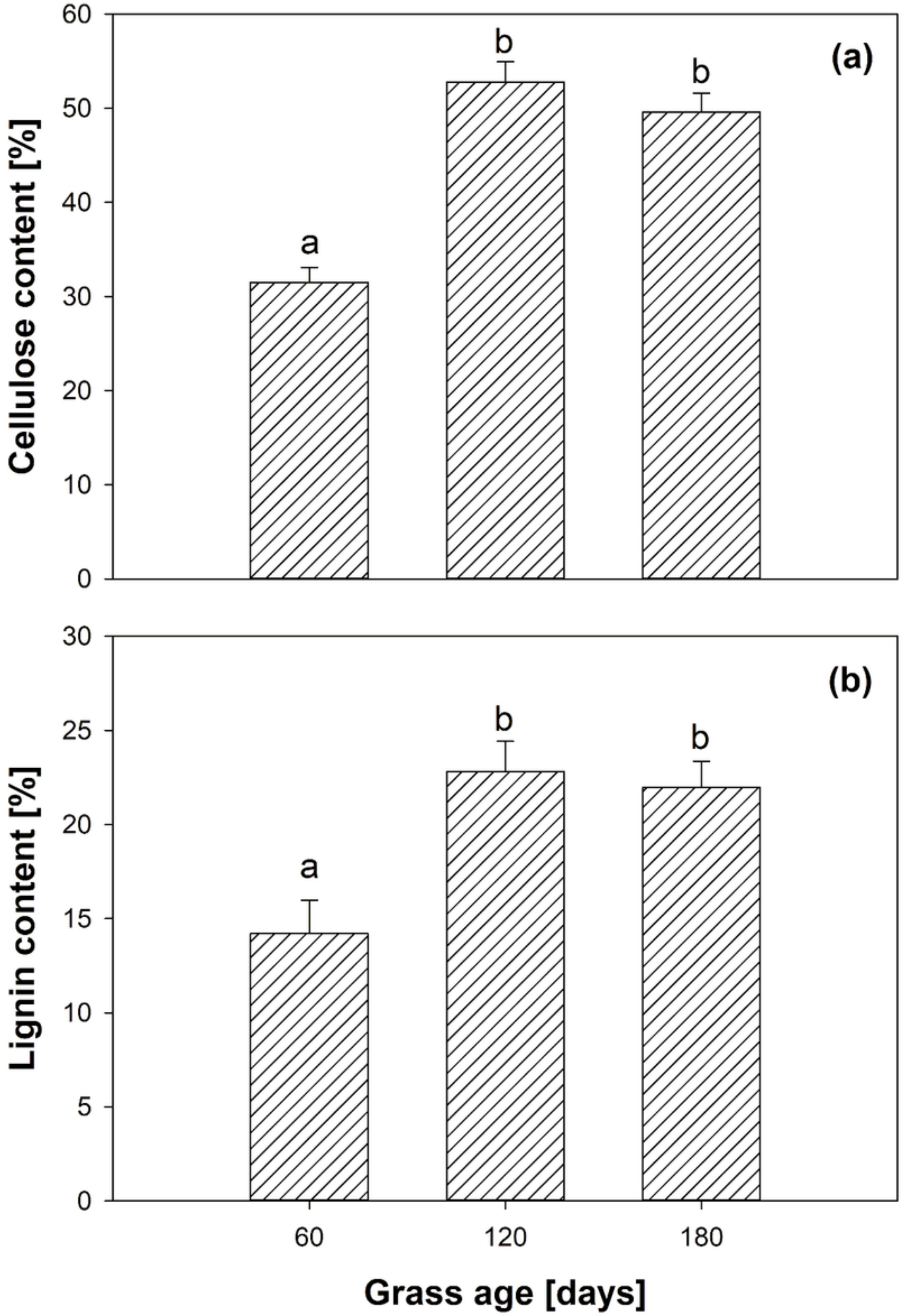

Figure 3

Mean (a) cellulose content and (b) lignin content (± standard error of mean) of C. dactylon roots randomly sampled from 60, 120 and 180-day-old grass. Letter indicates a statistically significant difference between treatments, as tested using one-way ANOVA followed by post hoc Tukey’s test.

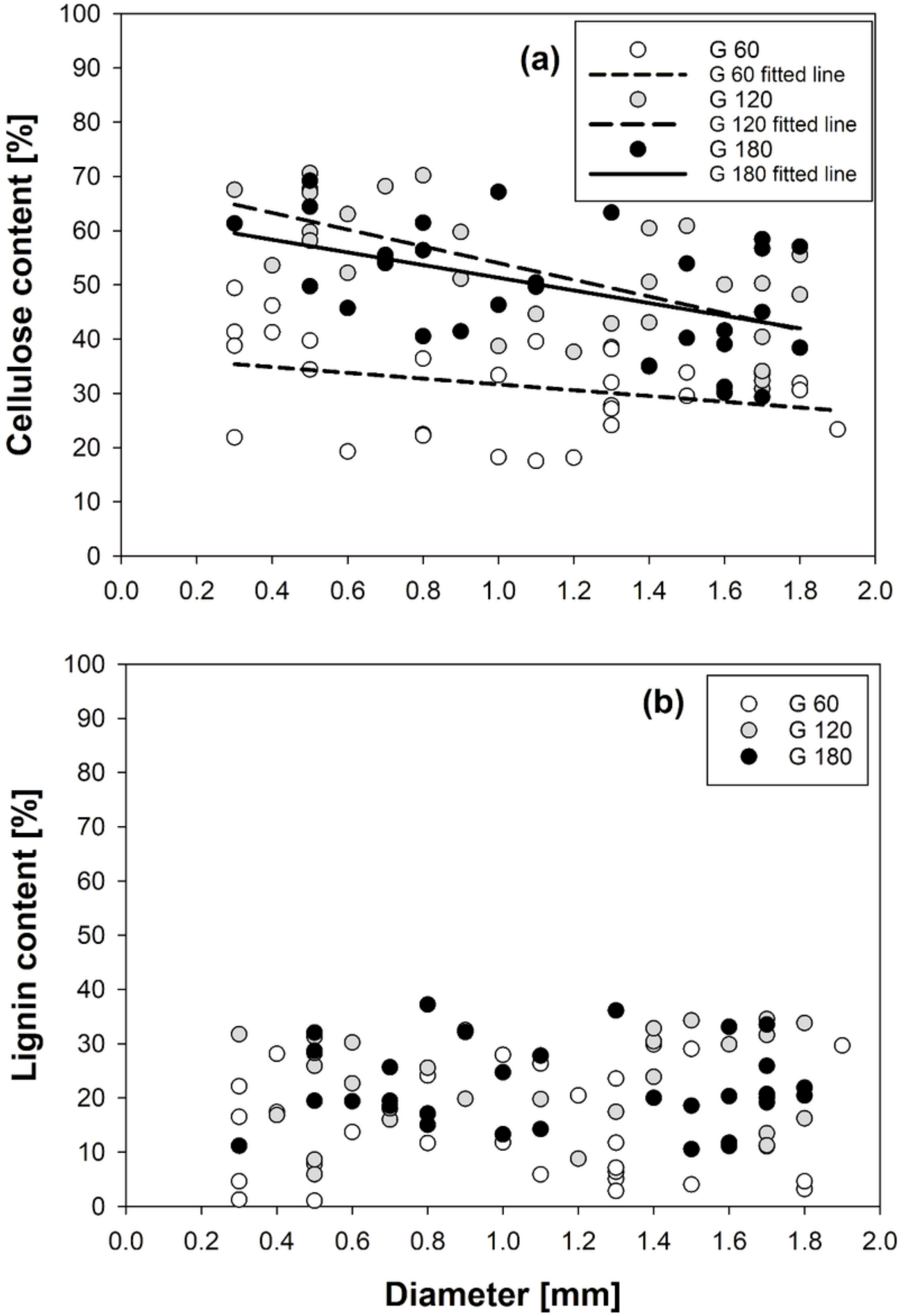

Figure 4

Relations between (a) cellulose content and (b) lignin content with root diameter of C. dactylon roots randomly sampled from 60, 120 and 180-day-old grasses. Solid lines represent the best-fitted curve. The fitting equations and goodness-of-fit (R2) are given in Table 2.

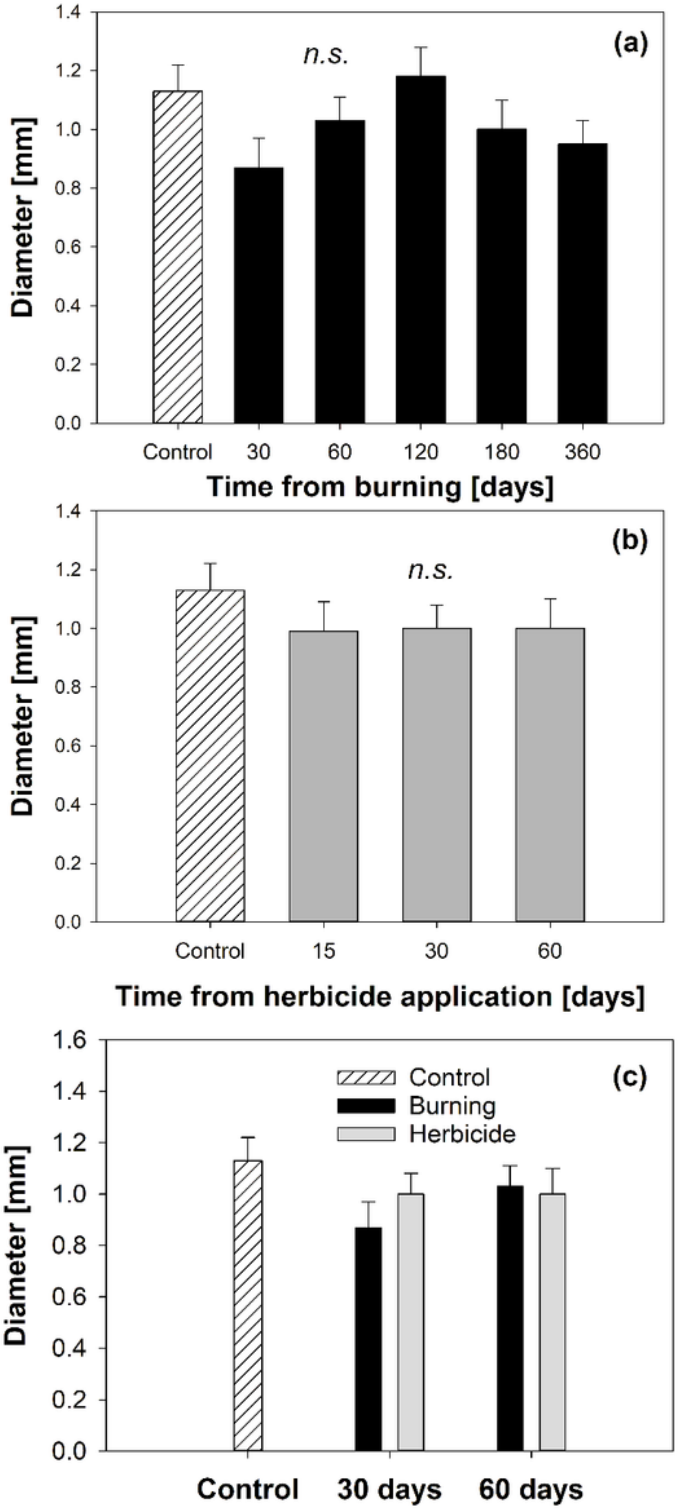

Figure 5

Mean root diameter (± standard error of mean) of Cynodon dactylon roots randomly sampled from planted soil columns (a) at 30, 60, 120, 180 and 360 days from grass burning; (b) at 15, 30 and 60 days from herbicide application; and (c) at 30 and 60 days comparing between the two grass-killing methods. Control (white bar with oblique pattern) gives the mean value recorded in 180-day-old plants (i.e.,

condition of plant before burning). n.s. indicates a non-statistically significant difference between treatments, as tested using one-way ANOVA.

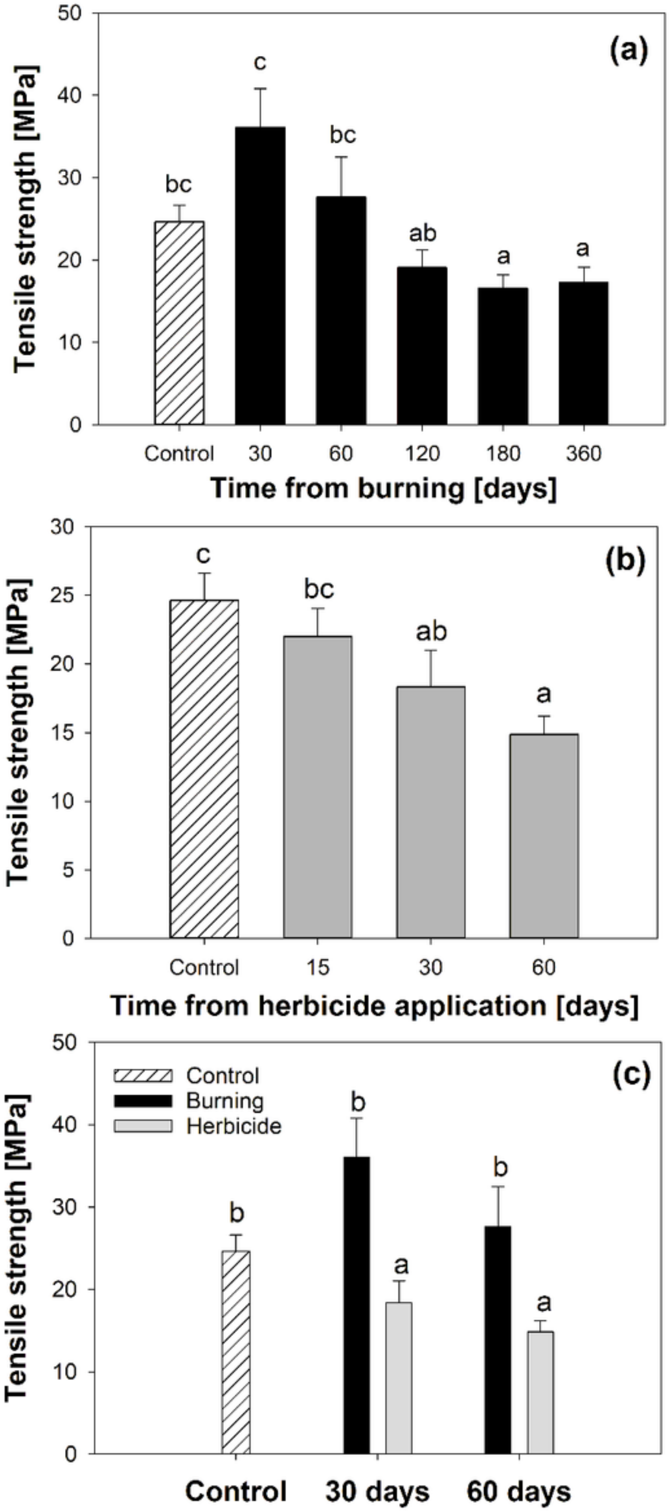

Figure 6

Mean tensile strength (± standard error of mean) of Cynodon dactylon roots randomly sampled from planted soil columns (a) at 30, 60, 120, 180 and 360 days from grass burning; (b) at 15, 30 and 60 days from herbicide application; and (c) at 30 and 60 days comparing between the two grass-killing methods.

Control (white bar with oblique pattern) gives the mean value recorded in 180-day-old plants (i.e., condition of plant before burning). Letters indicate a statistically significant difference between treatments, as tested using one-way ANOVA followed by post hoc Tukey's test. Data were log-transformed in statistical analysis.

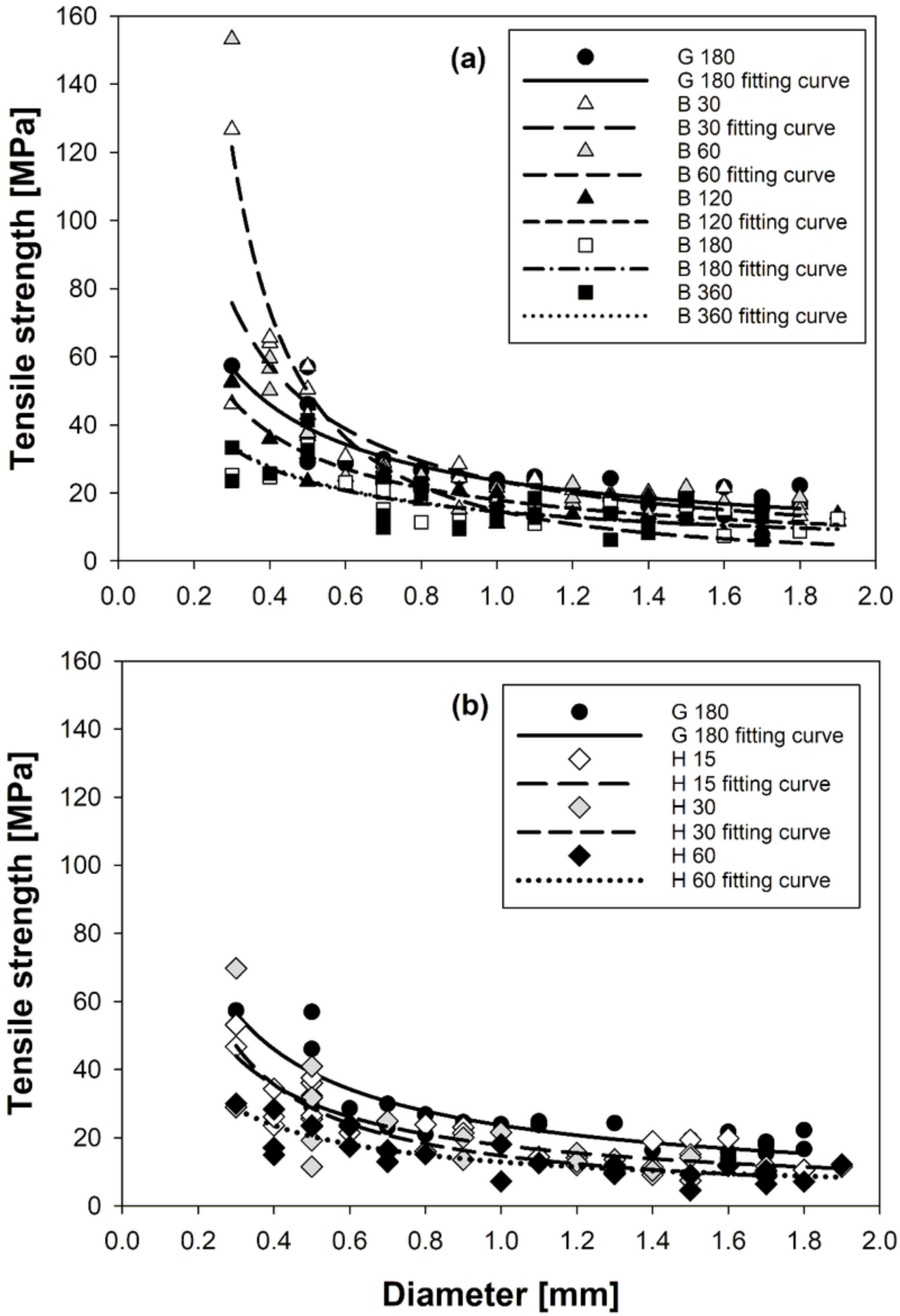

Figure 7

Relations between tensile strength and root diameter of Cynodon dactylon roots randomly sampled from planted soil columns (a) at 30, 60, 120, 180 and 360 days from grass burning; and (b) at 15, 30 and 60 days from herbicide application. Solid lines represent the best-fitted curve. The fitting equations and goodness-of-fit (R2) are given in Table 1.

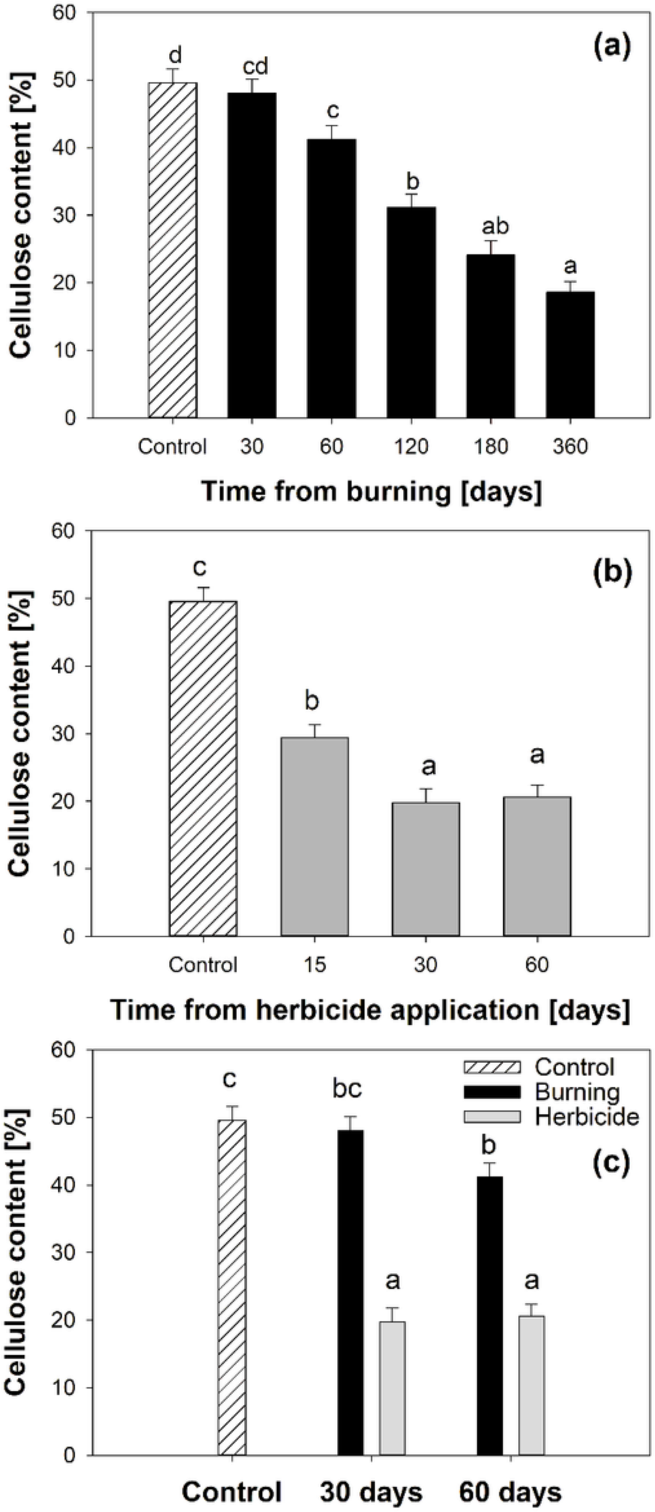

Figure 8

Mean cellulose content (± standard error) of Cynodon dactylon roots randomly sampled from planted soil columns (a) at 30, 60, 120, 180 and 360 days from grass burning; (b) at 15, 30 and 60 days from herbicide application; and (c) at 30 and 60 days comparing between the two grass-killing methods. Letter indicates a statistically significant difference between treatments, as tested using one-way ANOVA followed by post hoc Tukey's test. Data were log-transformed in statistical analysis.

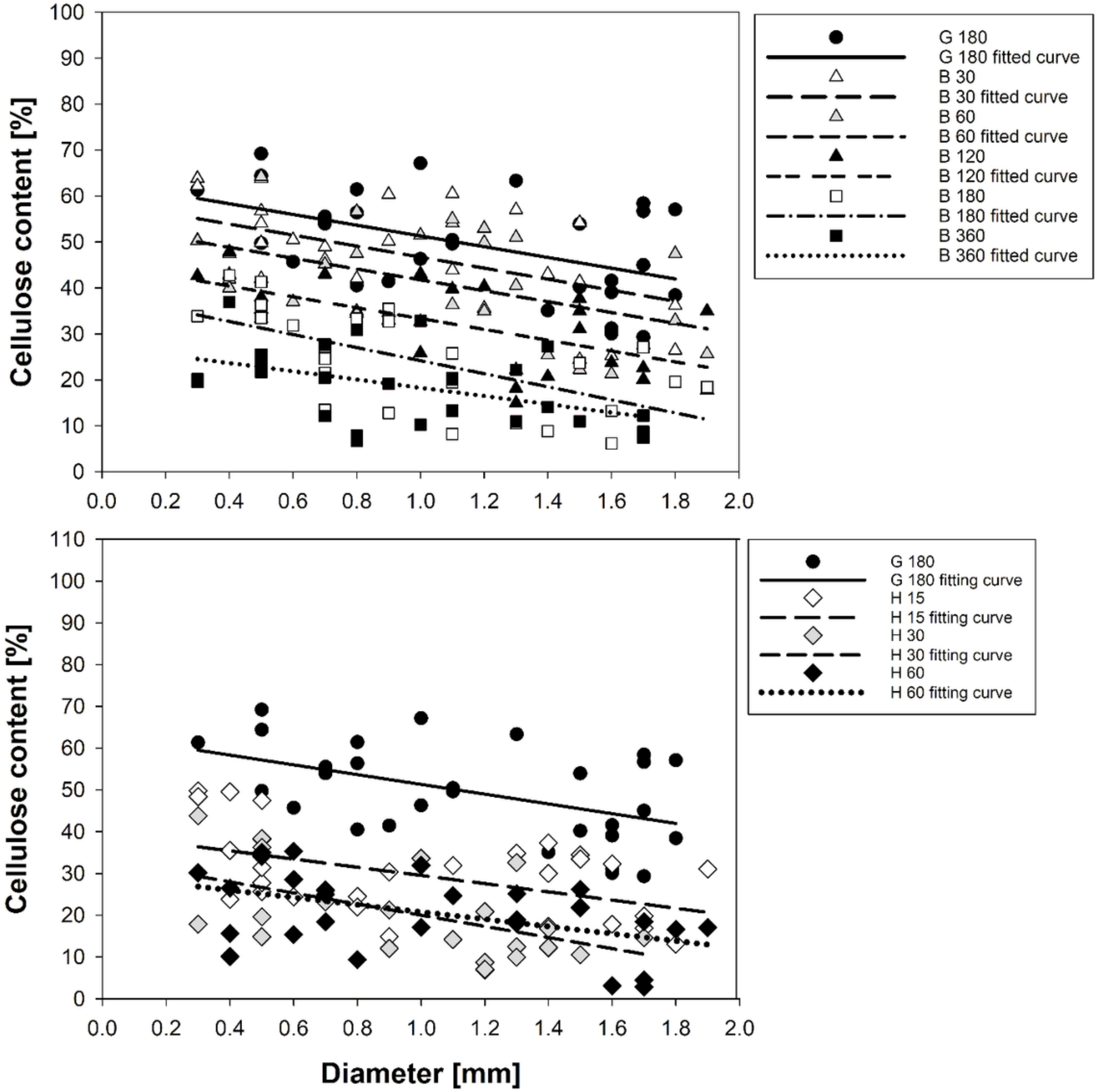

Figure 9

Relations between cellulose content and root diameter of C. dactylon roots randomly sampled from planted soil columns (a) at 30, 60, 120, 180 and 360 days from grass burning; and (b) at 15, 30 and 60

days from herbicide application. Solid lines represent the best-fitted curve. The fitting equations and goodness-of-fit (R2) are given in Table 2.

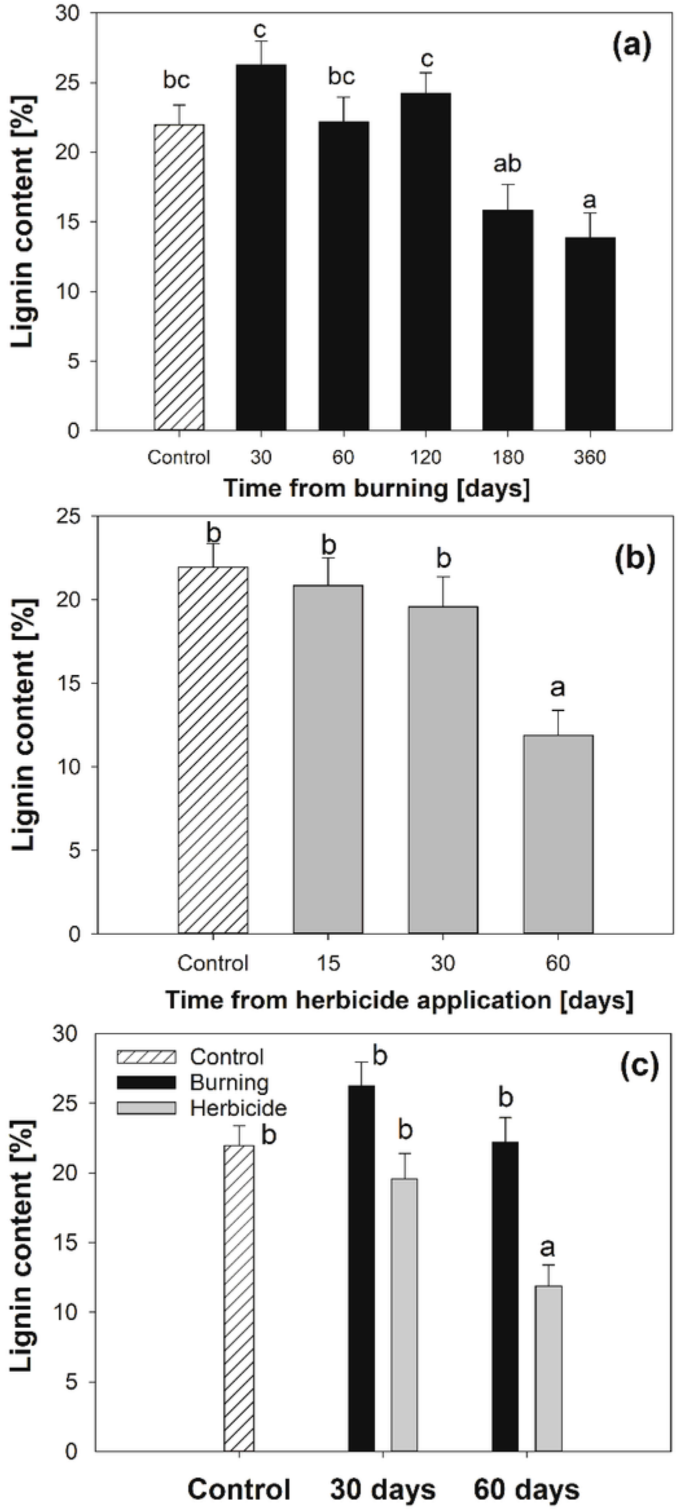

Figure 10

Mean lignin content (± standard error) of C. dactylon roots randomly sampled from planted soil columns (a) at 30, 60, 120, 180 and 360 days from grass burning; (b) at 15, 30 and 60 days from herbicide application; and (c) at 30 and 60 days comparing between the two grass-killing methods. Letter indicates

a statistically significant difference between treatments, as tested using one-way ANOVA followed by post hoc Tukey's test. Data were log-transformed in statistical analysis.

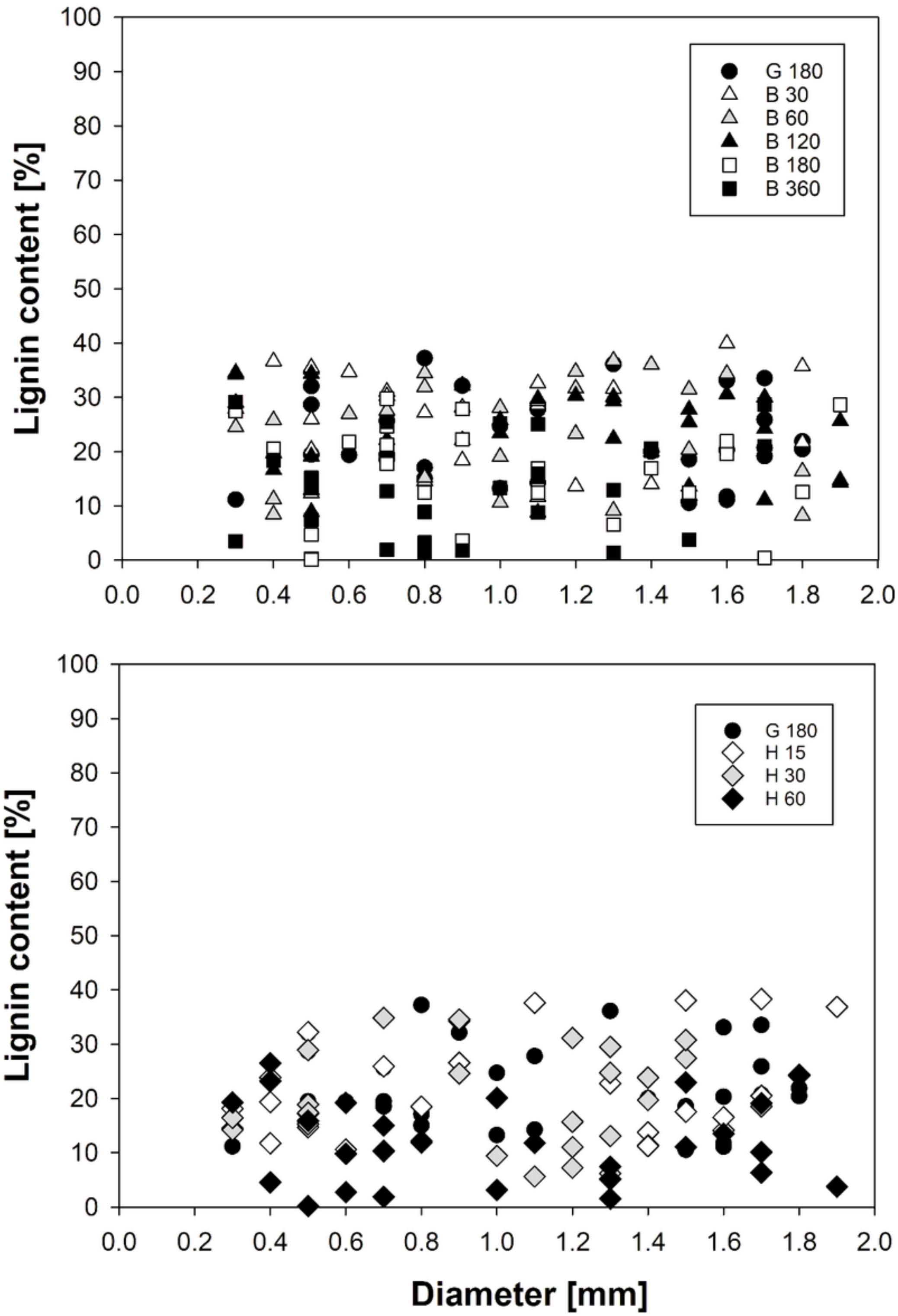

Figure 11

Relations between lignin content and root diameter of C. dactylon roots randomly sampled from planted soil columns (a) at 30, 60, 120, 180 and 360 days from grass burning; and (b) at 15, 30 and 60 days from herbicide application.