

**WOODY PLANTS IN DRYLANDS: PLASTIC
RESPONSES TO ENVIRONMENTAL STRESS**

Liang Xu

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Woody plants in drylands: plastic responses to environmental stress

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**Woody plants in drylands: plastic responses to
environmental stress**

**Struiken in droge gebieden: plastische reacties op
beperkende milieufactoren
(met een samenvatting in het Nederlands)**

**干旱区木本植物对环境胁迫因子的可塑性响应
(内含中文摘要)**

Proefschrift

ter verkrijging van de graad van doctor
aan de Universiteit Utrecht

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door

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To my families

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Chapter 1

General introduction

1. Serious ecological problems in dryland dune ecosystems

Land degradation and desertification in drylands

Drylands are regions with an aridity index, i.e. the ratio of annual precipitation to potential evapotranspiration, of less than 0.65, and they occur between 63°N and 55°S (Middleton & Thomas, 1997; Adeel *et al.*, 2005). Four subtypes of drylands are distinguished: dry sub-humid (aridity index from 0.50 to 0.65), semi-arid (0.20 – 0.50), arid (0.05 – 0.20) and hyper-arid (less than 0.05; Adeel *et al.*, 2005). Worldwide, drylands cover about 41% of the land regions of the Earth (Adeel *et al.*, 2005). The native ecosystems of these drylands face threats from global climate changes, e.g. decrease in rainfall, and from increasing human activity, e.g. overexploitation, inappropriate land use and human habitation (UNECA, 2007; Ravi *et al.*, 2010; Sonnenschein *et al.*, 2011). These factors result in serious land degradation that reduces or destroys the land's productivity and vegetation cover. Heavy degradation in drylands can lead to desertification, and presently large areas inhabited by more than 1 billion people are vulnerable to such deterioration (Squires, 2003; Adeel *et al.*, 2005; Verón & Paruelo, 2010). In general, expansion of the desert is a global problem (Adeel *et al.*, 2005; UNECA, 2007). Especially, China is one of the countries that severely suffer from desertification (Wu & Ci, 2002; Chen & Cai, 2003; SFAPRC, 2011). In China, about 27% of its total surface area (about 2.6 million km²) was under desertification by the end of 2009 (Wu & Ci, 2002; Chen & Cai, 2003; SFAPRC, 2011).

Common stress factors in dryland dune ecosystems

Plants in drylands are typically exposed to a suite of stress factors including: drought stress, grazing, sand burial, denudation, and wind exposure. The most obvious stress factor is drought stress induced by a strongly negative balance between precipitation and potential evapotranspiration. The generally uneven inter- and intra-annual precipitation and its heterogeneous distribution may aggravate this situation (Dong & Alaten, 1999; Mandujano *et al.*, 2007; Wang *et al.*, 2010b; Hanke *et al.*, 2011).

Drylands are increasingly being used for grazing livestock and with increasing human populations this form of land use is intensifying.

Overgrazing, the situation whereby the loss of plant tissue by animals grazing and browsing per growing season exceeds the regrowth potential of the vegetation, easily occurs in drylands as a result of low plant productivity (Li, 2010). Overgrazing is argued to be the major cause of desertification worldwide by affecting both plants directly and the surrounding environment, though other factors also play a role (Wu & Ci, 2002; Yoav *et al.*, 2006; Sørensen *et al.*, 2009). Desertification proceeds when the vegetation coverage of an area is strongly reduced, often as a result of overgrazing, and consequently the soil surface is exposed to strong wind and this may induce sand movement.

Sandland commonly consists of different types of dunes roughly defined according to vegetation cover, distinguishing mobile dunes (less than 15%), semi-fixed dunes (15% – 40%) and fixed dunes (more than 40%; Wang *et al.*, 2009a). Differences in vegetation cover are tightly related with sand mobility. Sand movement, usually occurs in mobile or semi-fixed dunes because there the top soil is in an unstable fixation stage. Sand movement may bring burial of aboveground plant parts or root baring by wind erosion. Burial, by wind-blown sand, directly damages leaves and dramatically changes the growth conditions of plants (Dech & Maun, 2006). Deep and long periods of burial impose conditions that lead to decreased plant survival and growth performances (Yu *et al.*, 2002; Dech & Maun, 2006).

Plants in dryland areas are frequently exposed to high levels of mechanical stress caused e.g. by animal trampling or drag forces imposed by the generally high wind speeds (Zhang, 1994; Wu & Ci, 2002; Xu *et al.*, 2012). This possibly contributes to reduction in growth and survival by increasing the risk of rupture of aboveground shoots. Exposure to mechanical stress is aggravated by the fact that the vegetation is generally sparse, resulting in a very limited degree of wind shielding (Wang *et al.*, 2008).

In drylands the above-mentioned stress factors typically occur simultaneously while each of these stress factors tends to have multiple effects. For example, low water availability can coincide with grazing, wind and sand movement. Grazing by large herbivores generally involves tissue losses, trampling and soil compaction. Strong wind involves mechanical stress, shifts in leaf microclimate and is the driving force of sand movement and thus indirectly causes burial or denudation (Zhang, 1994). Reduction in vegetation cover by overgrazing may aggravate nutrient loss in the top soil (van Staalduin & Werger, 2006). As discussed below these simultaneously occurring stressors often have interactive effects and may strengthen each other's impact on plants.

Nevertheless, many plant species commonly occur in drylands with varying degrees of sand mobility, indicating that they evolved mechanisms that enable them to tolerate these conditions. A fundamental understanding of the mechanisms that enable plants to tolerate the complex changes in environmental conditions that occur during the process of desertification will form an important basis for assessing desertification rates and planning restoration schemes.

2. Importance and adaption of woody species in dryland dune ecosystems

The crucial role of woody species in dryland dune ecosystems, an example from the sandland in northern China

The arid and semi-arid regions in northern China include large areas of the Gobi desert and semi-desert, spread over 18 provinces (autonomous regions, municipalities) located in the north and west of the country, including Beijing, Inner Mongolia, Ningxia, Shaanxi and Shanxi (SFAPRC, 2011). The plant life in these arid and semi-arid areas is surprisingly variable and includes annuals, perennial forbs, grasses and woody species (van Staalduinen & Werger, 2006; Bai *et al.*, 2008; Jin *et al.*, 2010). The abundance of various plant types correlates with the stability of the top soil and thus with the type of sand dune (Zhang, 1994; Li, 2001). The woody species predominantly are dwarf shrubs and small shrubs, while large shrubs and tree species are scarcer (van Staalduinen & Werger, 2006; Bai *et al.*, 2008; Jin *et al.*, 2010). These shrubs are believed to play a key role in controlling desertification and many of these species are used in vegetation restoration (Jiang & Li, 1977; Fang, 1983; Fu, 1993; Cui, 1998; Liu & Man, 2008). The woody species appear to have developed mechanisms that enable them to resist various forms of environmental stress, e.g. low evaporation rates, strong root systems, high tolerance to water and nutrient deficits, in these drylands, and apparently more so than grasses or herbs (Zhang, 1994; Li, 2001). Several of these species are important sources of fodder (Zhang, 1994).

In this dissertation I investigate these resistances of woody species in the Mu Us Sandland of northern China, which can be characterized as a semi-arid dryland. Many studies (Dong & Alaten, 1999; Yu *et al.*, 2002; Yu *et al.*, 2004; Liu *et al.*, 2006; Yu *et al.*, 2008; Wang *et al.*, 2009b) so far have focused on the effects of individual stress factors on these woody species. As noted above, however, focus on these individual effects may not be sufficient to fully understand how plants are adapted to drylands conditions. I have therefore particularly focused my study on the interactive effects of combined stress

factors (sand burial, defoliation, trampling, wind drag and drought).

At the population level the seedlings of perennial woody species run a high risk of death by external stress factors, e.g. long period of drought, frequent sand burial, overgrazing and mechanical impact, and these aspects have been studied in a complementary project by Li (2010). Li (2010) showed that survival of the seedlings until well into the reproduction stage is critical for population growth, and that species have developed particular life strategies to meet this challenge. My study complements this work by focusing on seedlings of not only the species used by Li (2010) but also other common woody species in the same area, and by analyzing the traits that underlie these life strategies. In so doing my thesis adds to our understanding of how woody plants are adapted to drylands conditions. Our complementary studies thus focused on the responses of seedlings (and later life stages) of local woody species to environmental stress.

Responses to differences in water content

In arid and semi-arid ecosystems, water is usually one of the most limiting factors for plant growth (Mandujano *et al.*, 2007; Wang *et al.*, 2009b; Hagiwara *et al.*, 2010; Wang *et al.*, 2010b; Hanke *et al.*, 2011). The physiological effects of water limitations on plants have been extensively reviewed in various textbooks (e.g. Lambers *et al.*, 2008) and include the effects of stomatal closure (reduced net CO₂ uptake and increased probability of damage in cell membranes) and turgor loss (effects on organ structure and functioning). To adapt to water stress, plants may enhance the ability of roots in hydraulic lift and of leaves in water use efficiency. Seed germination usually occurs after rainfall, and additional precipitation after germination is critical for the survival of seedlings (Huang & Gutterman, 2000; Fang *et al.*, 2006a). High water availability increases the growth of most species. Water availability may affect the biomass allocation to different plant parts. High water availability may lead to an increased allocation to shoots at the cost of roots, as well increases in for example leaf area per unit mass (Maherali & DeLucia, 2001; Ogaya & Penuelas, 2006; Wang *et al.*, 2009b).

The effects of changes in water availability may interact with other environmental conditions. For example, increase in allocation to stems is likely to increase stem rigidity, stem height and stem diameter which are associated with resistance to mechanical impact. Less allocation to roots probably increases the risk of mechanical stress as it affects the anchorage strength of a plant (Goodman & Ennos, 1996). The ability of plants to tolerate tissue loss through grazing has also been shown to depend on the level of

water availability, as that affects the period of photosynthetic activity, and thus growth, of the plant (see Wise & Abrahamson, 2007).

Responses to external mechanical stress

In dryland dune ecosystems, plants are commonly exposed to two different kinds of mechanical impact, wind drag imposed by the predominantly high wind speeds, and trampling by large herbivores (Wang *et al.*, 2008; Wang *et al.*, 2009b; Xu *et al.*, 2012). These forms of mechanical stress can directly rupture or damage plant parts, which can be lethal, or may indirectly cause reductions in growth and/or reproduction (Ennos, 1997). Exposure to mechanical stress typically induces changes in plant traits known as thigmomorphogenesis (Jaffe, 1973), including stem height and basal diameter (Telewski, 1990; Jaffe & Forbes, 1993; Sun & Liddle, 1993a; Roberto, 1999; Henry & Thomas, 2002; Anten *et al.*, 2005; Andrés-Abellán *et al.*, 2006; Hill & Pickering, 2009; Hill & Pickering, 2009; Xu *et al.*, 2012), root production (Crook & Ennos, 1994; Niklas, 1998; Henry & Thomas, 2002; Anten *et al.*, 2006), leaf characteristics (Sun & Liddle, 1993a; Liu *et al.*, 2007b; Wang *et al.*, 2008; Wang *et al.*, 2009b; Anten *et al.*, 2010), seed size (Meerts & Garnier, 1996) and flexural stiffness of stems and roots (Telewski, 1994; Goodman & Ennos, 1996; Anten *et al.*, 2005; Liu *et al.*, 2007b; Wang *et al.*, 2008; Wang *et al.*, 2009b; Xu *et al.*, 2012). These responses are believed to be adaptive in mechanical stressful environments because they increase the ability of plants to resist mechanical stress (Ennos, 1997; Anten *et al.*, 2005).

The mechanical effect of wind drag and trampling on plants however is different. Wind usually acts on all aboveground parts of a plant while trampling acts on one point. The force imposed by wind is usually small compared with the overwhelming force of trampling. As a result, plants may have evolved different strategies of resistance to wind and trampling. In general plants can prevent mechanical damage either by increasing the strength of their support organs (e.g. stems) to resist breakage or by producing more flexible shoots to reconfigure relatively easily and thus minimize the forces encountered (Puijalón *et al.*, 2008; Paul-Victor & Rowe, 2011; Puijalón *et al.*, 2011). These two strategies have been associated with stress tolerance and stress avoidance, respectively (Puijalón *et al.*, 2008). A trade-off between strength and flexibility can be expected because thicker structures are stronger but less flexible than thinner structures (Puijalón *et al.*, 2011). In the case of wind, both strategies are feasible, i.e. plants can either make strong support structures that resist wind forces or flexible structures that avoid them (Butler *et al.*, 2011). Trampling, on the other hand, entails overwhelmingly large

forces, such that stem stress avoidance through increased flexibility is the only viable response for small or young plants. However, while there has been much work done on mechanical traits in relation to wind resistance, little similar work has been conducted on trampling, most of which has focused on grasses (Sun & Liddle, 1993a; Sun & Liddle, 1993b; Engelaar & Blom, 1995; Kobayashi *et al.*, 1999; Littlemore & Barker, 2003; Rodgers *et al.*, 2003; Wang *et al.*, 2008; Hill & Pickering, 2009).

Response to sand burial

Effects of burial on plants depend on the rate and depth at which it occurs. Small amounts of burial usually have no or only slight effects on plants while deeper burial acts as a stressor (Maun, 2004; Li *et al.*, 2010a, b). A direct effect of burial is that leaves are being covered and can therefore no longer photosynthesize. Burial also affects the conditions in the soil, e.g. through reductions oxygen availability to roots, thus impacting their function, and through changes in soil temperature and moisture (Yu *et al.*, 2002; Dech & Maun, 2006). Key responses to burial include the production of new leaves, elongation of stems and the formation of adventitious roots (Brown, 1997; Dech & Maun, 2006; Gilbert & Ripley, 2008), which lead to morphological and physiological changes in leaves, stems and roots (Lytle & Poff, 2004). This response is probably partly facilitated by a remobilization of resources from buried leaves (Gilbert & Ripley, 2008). More investment to leaves partly compensates for the loss in photosynthetic area. As burial beyond a certain threshold may be fatal, fast shoot elongation is important, as it exposes the stem above the soil and maintains sufficient space for new leaf production. Adventitious roots in response to burial provide access to more water, nutrients and oxygen to support growth (Maun, 2004; Dech & Maun, 2006).

Different trait responses to burial may be functionally coordinated. Rapid shoot elongation possibly brings potential mechanical rupture to aboveground stems (Niklas, 1992). Sand burial itself may provide partial mechanical support to the stem. Production of adventitious roots may increase the anchorage strength of plants (Goodman & Ennos, 1996), which can partially mitigate mechanical risk to a more vulnerable stem caused by increased stem elongation. Moreover, it is expected that the basal diameter or stem stiffness should increase with shoot elongation to keep the stem stable.

Responses to defoliation

Many woody species are browsed by livestock as forage (Jiang & Li, 1977; Fang, 1983; Fu, 1993; Cui, 1998; Hegland *et al.*, 2010). Browsing (and

grazing) is a complex process that induces several effects on plants, two important of which include defoliation and the associated reduction of total photosynthetically active leaf area (Hanley & Sykes, 2009; Gruntman & Novoplansky, 2011), and trampling which brings mechanical stress to the plant (Sørensen *et al.*, 2009). Trampling effects have been discussed above and here I focus on defoliation. The defoliation due to grazing or browsing directly causes loss of resources and reduction in photosynthetic surface that can be mitigated by compensatory growth of plants (Anten *et al.*, 2003; Gruntman & Novoplansky, 2011; Muola *et al.*, 2011; Zhang *et al.*, 2011). Typically plants respond to defoliation by increasing allocation to leaves at the expense of allocation to roots and stems (Anten *et al.*, 2003; van Staaldin & Anten, 2005; Stevens *et al.*, 2008). Compensatory growth can mitigate potential negative effects of defoliation (Anten *et al.*, 2003; van Staaldin & Anten, 2005) and stored carbohydrates may be used to support growth after defoliation (McPherson & Williams, 1998).

Effects of clonal growth on stress alleviation

A large majority of the species in dryland dune ecosystems are clonal. Clonal species are characterized by their ability to produce vegetative offspring (ramets) often along horizontal stems (stolons) or roots (rhizomes). This clonal growth form is regarded as an important mechanism to exploit the resources available at a given site or colonize adjacent sites (Hutchings & de Kroon, 1994; Dong & Alaten, 1999; Liu *et al.*, 2006; Yu *et al.*, 2008; Beatty & Provan, 2011; Gruntman & Novoplansky, 2011; Sui *et al.*, 2011). Ramets of clonal plants may grow in small patches with different availability of resources. Connections between these ramets by rhizomes, stolons allow exchange of water, nutrients, carbohydrates and other resources to replenish local shortage of resources. This is known as intra-clonal division of labor (Stuefer *et al.*, 1996; Yu *et al.*, 2004; Roiloa *et al.*, 2007; Li *et al.*, 2008; Yu *et al.*, 2008; de Witte & Stöcklin, 2010). Clonal exchange can alleviate the negative effects of many stress factors, also in dryland shrubs, e.g. where plant parts are buried or roots are exposed. Therefore, I address the hypothesis that clonal integration helps to elevate plant tolerance to mechanical stress and other multiple combinations of stresses.

3. This study

Study purposes

As discussed above plants in drylands are exposed to a suite of stress factors

which may have strongly interactive effects. Woody plants are an important component of drylands vegetation and appear to exhibit traits that make them particularly well-adapted to these conditions. Moreover many woody species are used in combating desertification. This thesis focuses on the growth, phenotypic and mechanical characteristics and eco-physiology of woody species in Mu Us Sandland, Inner Mongolia, China, in response to different stress factors mentioned above. Unlike most previous studies on this issue, I place particular emphasis on the interactive effects of different stress factors. Particularly, the objectives of this thesis include:

- 1) a study of how woody species respond to local stress;
- 2) investigations on the interactive effects of these stress factors on plants;
- 3) evaluation of the role of clonal growth in alleviating the effects of these stress factors;
- 4) a deepening of the understanding of inter- and intra-specific variation in adaptive responses of the plants.

Study sites

In China, I conducted experiments at the Ordos Sandland Ecological Research Station (OSES, 39°29'37.6"N, 110°11'29.4"E, 1300 m a.s.l.) of the Institute of Botany, the Chinese Academy of Sciences, located in Mu Us Sandland in Inner Mongolia (Fig. 1a).

The Mu Us Sandland lies in the agro-pastoral transitional zone in northern China, occupying an area of about 40,000 km² (Wu & Ci, 2002). The main vegetation in Mu Us Sandland is sandy grassland (Zhang, 1994; Wu & Ci, 2002). I collected seeds and did field experiments near the station. The average summer temperature there is 20 – 24 °C, and the average annual precipitation is 260 – 450 mm, mainly in the period from July to September (Zhang, 1994). Due to severe desertification in the past decades, this area, which used to be typical grassland (steppe), is now covered by different types of sandland, including fixed, semi-fixed and mobile dunes (Zhang, 1994). This desertification is due to the persistent overgrazing in this area (Huang *et al.*, 2000). In the dunes where I did field experiments and collected seeds a number of woody species commonly occur: *Artemisia ordosica*, *Caragana intermedia*, *Cynanchum komarovii*, *Hedysarum laeve* and *Populus simonii*.

Research based on meteorological data from 1961 to 2007 (Wang *et al.*, 2010a) found an increasing trend in temperature but a decreasing trend in precipitation in Mu Us Sandland. These changes in climate contribute to the transformation of fixed dunes to semi-fixed dunes with its associated change in vegetation (Wang *et al.*, 2010a). Increasing human activity in the form of

land exploitation, overgrazing, and misuse of water has accelerated the rate of desertification (Wu & Ci, 2002).

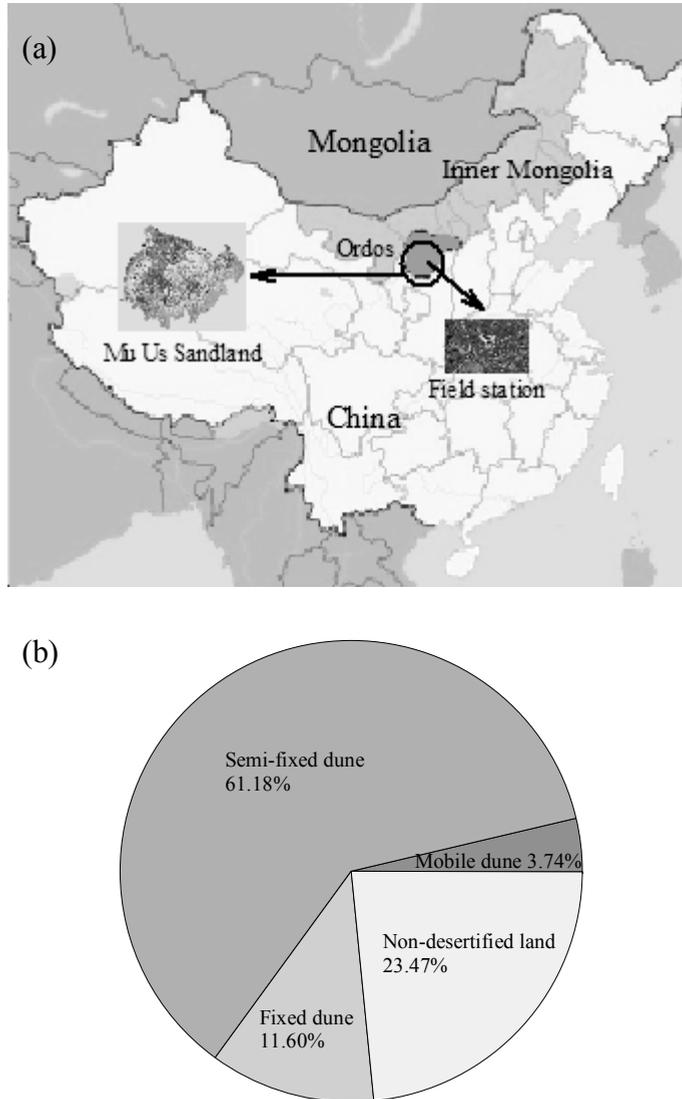


Fig. 1. Map showing the approximate extent of Mu Us Sandland and position of the field station in China (a) and composition of land types of Mu Us Sandland in 2006 (b). Data is from the remote sensing dynamic monitor and early warning system of Mu Us Sandland (<http://www.nmgygzx.com>).

In the Netherlands, I worked in the climate-controlled and plastic greenhouse compartments located at the Utrecht University Botanical Gardens (52° 5' 16.79"N, 5° 10' 8.26"E).

Focal species

Six woody species were used in this study. Some species i.e. *Artemisia ordosica*, *Caragana intermedia* and *Hedysarum laeve* were used by Li (2010) in her study of population dynamics. This research at the level of individual traits complements her work. These species are important for vegetation restoration or livestock forage. Clonal rhizomatous plants are suitable for dune fixation in early fixation stages. Non-clonal plants with high survival, fast growth and reproduction can be used for dune fixation after the sandland is partly fixed (Li, 2010).

Table 1. Dominant habitat dune types and growth form of focal woody species used in this thesis.

Species	Growth type			Dune type	
	Clonal	Non-clonal	Mobile	Semi-fixed	Fixed
<i>Artemisia ordosica</i>		×		×	×
<i>Caragana intermedia</i>		×		×	×
<i>Cynanchum komarovii</i>		×		×	×
<i>Hedysarum laeve</i>	×		×	×	
<i>Hippophae rhamnoides</i>	×		×	×	
<i>Populus simonii</i>	×		×	×	

Artemisia ordosica Krasch (Asteraceae) is a shrub with a long thick main root and many lateral roots. It can produce adventitious roots under burial (Zhang *et al.*, 2010). The main shoot usually reaches a height of 50-100 cm and branch length commonly is 10 – 35 cm. Old wooden branches are dark grey or dark dust-colored but the younger branches are fuchsia-reddish. It flowers and fruits from July to October (Lin & Lin, 1991). This species is widely distributed in the semi-fixed and fixed dunes in the semi(arid) region of North China and Mongolia. Plants usually are recruited by reproduction from seeds (Huang & Gutterman, 2000; Li, 2010). As one of the dominant shrub species, it can fix mobile sand, reduces wind speed and actively alleviates desertification (Xu *et al.*, 2007).

Caragana intermedia Kuang et H. C. Fu (Fabaceae) is a perennial deciduous shrub species, and is widely distributed in the drylands of northern China (Xiao *et al.*, 2003). The deep root system can extend over six meters in

depth (Fang *et al.*, 2006a). Shrubs typically are 70-150 cm tall and have compound, plumose leaves (Fu, 1993). Old wooden branches are yellowish or grey-green. Flowers occur in May and seeds ripen in July (Fu, 1993). The species can endure coldness, drought, sand burial and strong winds. It is an important fodder plant (Guo *et al.*, 2010).

Cynanchum komarovii Al. Ijinski (Asclepiadaceae) is a perennial semi-shrub with leathery, opposite, narrowly elliptic leaves (Jiang & Li, 1977). It is highly tolerant to drought and high temperatures, and is regarded as one of the indicator plants of heavy desertification. Plants grow to a height of 50 cm. They do not produce adventitious roots under burial. It usually flowers from June to August and fruits from July to September. Although stems and leaves are a little poisonous to livestock, *C. komarovii* is nevertheless consumed in the dry season when forage is short (Chen & He, 2006). This species is also an important nectar source in Mu Us Sandland (Chen & He, 2006).

Hedysarum laeve Maxim. (Fabaceae) is a clonal shrub species that easily propagates vegetatively by horizontal rhizomes (Zhang *et al.*, 2001). It flowers profusely and seeds easily germinate. These two modes of reproduction allow this species to successfully dominate the mobile and semi-fixed dunes (Liu *et al.*, 2007a; Li, 2010). Stems of adult plants are usually 1 – 2 m high and bear compound leaves (Cui, 1998). It usually flowers from late June to middle September and fruits ripen in October or November. As a result of the high reproductive capacity and tolerance to drought, this species is used to stabilize moving sand. It also is an important and favourite fodder plant in the drylands (Cui, 1998).

Hippophae rhamnoides L. (Elaeagnaceae) is a deciduous shrub with some stem thorns and with sub-opposite, silvery-grey leaves. Plant height commonly varies from 1 – 5 m. It flowers from April to May and fruits from September to October (Fang, 1983). The species is adapted to the dry environment as it is strongly clonal, and appears to have a strong physiological integration (He *et al.*, 2006). Root systems extend both horizontally and vertically to form strong networks (He *et al.*, 2006). The species is used in windbreaks and to fix sand (Fang, 1983). Its berries are used in fruit juice and as a medicine, its leaves as forage and its stems and roots as fuel (He *et al.*, 2006).

Populus simonii Carr. (Salicaceae) is a tree that can reach a height of 20 m and a stem diameter of 0.5 m (Wang & Fang, 1984). As it is highly resistant to cold, drought and wind, and it has a wide distribution across many provinces in northern China. *P. simonii* is a root-suckering clonal species whose roots

extend horizontally for many meters; new ramets are produced on these roots. The small ramets from horizontal roots are often grazed by animals. The thick horizontal roots also generate small fibrous roots. In mobile dunes, the roots are easily damaged by exposure to the dry air when they are denuded, or by trampling. It is an important local species used for the stabilization of mobile dunes and the recovery of vegetation in many parts of northern China (Liu & Man, 2008).

H. laeve, *H. rhamnoides* and *P. simonii* are clonal plants while *A. ordosica*, *C. intermedia* and *C. komarovii* are non-clonal. All these species commonly occur on mobile and fixed dunes. However, dominance of these species varies according to dune type (Table 1).

Support

This study is part of a collaborative project within the existing Joint Research Project of the Chinese Academy of Sciences (CAS) and the Royal Netherlands Academy of Arts and Sciences (KNAW). The Joint Research Project started in 1999 and my study lasted from 2008 to 2012. It is the complementary project of project 06-PhD-01 (Shouli Li) focusing on the population dynamics of woody plants in Inner Mongolia, China. The work in these projects was done within the framework of a long-term cooperation between the Department of Ecology and Biodiversity, Utrecht University (UU), and the Institute of Botany, the Chinese Academy of Sciences (IBCAS), Beijing. The research comprised comprehensive censuses and many greenhouse and field experiments and resulted in several scientific papers published in international journals.

4. Outline of this dissertation

Chapter 1 gives a general description of my research questions and their relevance.

In Chapter 2 I test how simulated wind force (brushing), sand burial and defoliation affect the growth and mechanical properties of *Cynanchum komarovii*.

In Chapter 3 I investigate whether physiological integration helps the root-suckering clonal plant *Populus simonii* to alleviate damage caused by trampling and removal of leaves, the two main effects associated with grazing.

Chapter 4 deals with the variations in growth and mechanical traits of four woody species in response to interaction of different water regimes and trampling.

Chapter 5 describes a sand burial experiment conducted on seedlings of

Caragana intermedia, belonging to 18 seed families. There I investigated the variation in phenotypic plasticity within that species.

The final chapter, Chapter 6, integrates all the results and gives a general discussion on how woody species are adapted to the complex pattern of stress conditions that prevail in dry sandlands.



Chapter 2

Interactive effects of mechanical stress, sand burial and defoliation on growth and mechanical properties in *Cynanchum komarovii*

with Fei-Hai Yu, Marinus J. A. Werger, Ming Dong and Niels P. R. Anten

Abstract

- In drylands wind, sand burial and grazing are three of the important factors affecting growth and mechanical properties of plants, but their interactive effects have not yet been investigated.
- Plants of the semi-shrub *Cynanchum komarovii*, common in semi-arid parts of NE Asia, were subjected to brushing, burial, and defoliation. We measured biomass allocation and relative increment rates of dry mass (RGR_m), height (RGR_h) and basal diameter (RGR_d), respectively. We also measured stem mechanical properties such as, the Young's modulus (E), second moment of area (I), flexural stiffness (EI), breaking stress (σ_b), and scaled these traits to the whole-plant level to determine the maximum lateral force ($F_{lateral}$) and the buckling safety factor (BSF).
- Brushing increased RGR_m . Neither burial nor defoliation independently affected RGR_m , but together they reduced it. Among buried plants, brushing positively affected stem rigidity and strength through increasing RGR_d , E , I and EI and at whole plant this resulted in a larger BSF and $F_{lateral}$. However among unburied plants this pattern was not observed.
- Our results thus show that effects of mechanical stress and grazing on plants can be strongly modified by burial, and these interactions should be taken into account when considering adaptive significance of plant mechanical traits in drylands.

Key words: Brushing, burial, drylands, grazing, relative growth rate, thigmomorphogenesis.

Introduction

Plants in arid and semi-arid regions are frequently exposed to high levels of mechanical stress (MS hereafter) caused e.g. by wind, or animal trampling, sand burial and grazing (Zhang, 1994; Wu & Ci, 2002). Wind load-up can be particularly prevalent as in these areas wind speeds tend to be relatively high and plants tend to grow solitarily and thus do not shield each other from wind (Wang *et al.*, 2009). Trampling can also be common in areas where grazing occurs. MS is among the most important environmental factors that strongly contribute to the development of plant morphological and anatomical characteristics (Ennos, 1997). Exposure to MS induces changes in plant traits, including stem height and basal diameter (Telewski, 1990; Jaffe & Forbes, 1993; Roberto, 1999; Henry & Thomas, 2002; Anten *et al.*, 2005), root production (Crook & Ennos, 1994; Niklas, 1998; Henry & Thomas, 2002; Anten *et al.*, 2006), leaf number (Liu *et al.*, 2007b; Wang *et al.*, 2008; Wang *et al.*, 2009b), and flexural stiffness of stems and roots (Telewski, 1994; Goodman & Ennos, 1996; Anten *et al.*, 2005; Liu *et al.*, 2007b; Wang *et al.*, 2008, 2009b). These responses to MS, termed thigmomorphogenesis (Jaffe, 1973; thigmo hereafter), are believed to be adaptive in mechanical stressful environments because they increase the ability of plants to resist mechanical stress (Ennos, 1997; Anten *et al.*, 2005).

Plants in dry areas are exposed to many other environmental stress factors in addition to MS, such as water shortage, sand burial and defoliation. The effects of these factors on plants may interact with those of MS. Although studies show that shading (Holbrook & Putz, 1989), nutrient availability (Grace *et al.*, 1982) and water supply (Retuerto & Woodward, 1993; Wang *et al.*, 2009b) can modify plant responses to MS, to our knowledge, interactive effects of MS with defoliation and sand burial have not yet been investigated.

In many dry areas strong wind causes sand movement and can cause plants to be buried (Zhang, 1994). This can lead to a reduction in oxygen supply to roots and reduction of photosynthetic area of the plants (Dech & Maun, 2006). Plants typically respond to moderate levels of sand burial through increased stem elongation (Brown, 1997) and the formation of adventitious roots (Dech & Maun, 2006). Moreover, sand burial may provide partial mechanical support to the stem and increase the anchorage strength of plants (Goodman & Ennos, 1996). While increased stem elongation may make plants more vulnerable to mechanical damage (Niklas, 1992), the greater anchorage strength may mitigate this effect. But even though exposure to wind drag force and sand burial are correlated environment factors, no study that we know of

has investigated whether and in what direction burial may modify the effects of MS.

Overgrazing is another important stress factor in the Mu Us Sandland and is a primary cause of vegetation collapse and desertification in many drylands (Wu & Ci, 2002). Defoliation due to grazing can greatly reduce photosynthetic tissues, and plants typically respond to defoliation by increasing biomass allocation to leaves and decreasing that to roots (Stevens *et al.*, 2008). Therefore, the effects of defoliation on biomass allocation tend to be in the opposite direction as the effects of MS (Caldwell, 1970; Henry & Thomas, 2002). In addition, a decrease in leaf area by defoliation can directly reduce wind drag on plants. We therefore hypothesize that defoliation can modify the effects of MS on plants. In addition, since both defoliation and burial entail losses in exposed leaf area we also hypothesize that one aggravates the effects of the other. Overall our understanding of plant adaptations to multiple stress factors such as those that occur in drylands entails the use of controlled experiments that clearly investigate the individual and interactive effects of these factors.

To test our hypotheses we conducted a quantitative greenhouse experiment in which plants of the semi-shrub *Cynanchum komarovii* were subjected to two levels of brushing (with or without brushing to simulate the presence or absence of MS; standardized using a brushing machine), two levels of sand burial (with or without burial) and two levels of defoliation (with or without shoot removal) in a factorial design. We recognize that wind may also change microclimatic effects in addition to MS, but in this paper we limit ourselves to potential interaction among MS, burial and defoliation. Specifically, we addressed the following questions: (1) What are the effects of MS, sand burial or defoliation on growth, morphology and mechanical properties of *C. komarovii*? (2) Are the effects of these three stressors additive or are there interactions? (3) What are the consequences of changes in mechanical traits for whole plants mechanical stability?

Material and Methods

The species

Cynanchum komarovii Al. Iljinski (Asclepiadaceae) is a non-clonal perennial semi-shrub widely distributed in the Mu Us Sandland (Jiang & Li, 1977). It is highly tolerant to drought and high temperatures, and regarded as one of the indicator plants of heavy desertification. The single stem of this caespitose species can grow to a height of 50 cm. Leaves are leathery, opposite and

narrow-elliptic. The stem and fiber roots come from the underground tuber. The stem does not produce adventitious roots under burial. *Cynanchum komarovii* usually flowers from June to August and fruits from July to September. Although the stem and leaf are a little poisonous to livestock, *C. komarovii* is still consumed in the dry season when the forage is short (Chen & He, 2006). This species is also an important nectar source in the Mu Us Sandland (Chen & He, 2006).

Study site

The experiment was conducted in a greenhouse at Ordos Sandland Ecological Research Station (OSES, 39°29'37.6"N, 110°11'29.4"E, 1300 m a.s.l.) of the Institute of Botany, Chinese Academy of Science, located in the Mu Us Sandland in Inner Mongolia Autonomous Region, China. The average annual temperature is 7.5–9.0 °C and average annual precipitation is 260–450 mm (Zhang, 1994). This area previously was typically covered by grasslands, but is now dominated by sandland, consisting of fixed, semi-mobile or mobile dunes and inter-dune lowlands. Plants in this area are exposed to high levels of wind during the whole year, especially in spring (Zhang, 1994).

Experimental design

On 19 July 2009, 72 *C. komarovii* seedlings of similar size were collected near OSES and planted in pots (21 cm in height and diameter) filled with local sand collected around OSES in the greenhouse. As a result of the poor growth after transplantation, some plants were discarded. After 10 days of growth, seven seedlings were randomly selected and harvested to determine the initial biomass distribution at the initiation of the treatments. Of the remaining seedlings, 56 were randomly and equally assigned to eight treatments, consisting of two levels of mechanical stress treatments (no brushing *vs.* brushing), two levels of sand burial (no burial *vs.* burial) and two levels of defoliation (no clipping *vs.* clipping) in a factorial design. For the burial treatment, four thin wooden sticks of 20 cm were vertically inserted into the sand until they reached the inner walls of pots. A transparent plastic belt was wrapped around the sticks. The belts were carefully filled with sand (from the same stock as the sand used to fill the pots, see above) so that plants were buried in sand to a depth of 50% of their stem height. For the defoliation treatment 50% of the leaves were removed by clipping one of every two leaves along the stems. In the treatments in which plants were both buried and defoliated we first defoliated and then buried the plants. For the plants without sand burial, plastic belts were also installed to prevent potential confounding

effects of modifying the microclimate. Positions of pots were randomly changed every two weeks during the experiment.

The brushing treatments were conducted by a machine. The machine was shaped like a cube with four wheels. A beam of 2 m long was fixed on two chains. The chain which was at the side of the machine connected with the erect lifting shaft arm. This setup allowed to adjust the brushing height. Rotational frequency of the chain, driven by an engine, was controlled by a panel. Rotational speed could be calculated from the rotational frequency and circumference of the chain. One-time-folded printing papers were fixed on the beam with clips. The rotary beam with papers was long enough to touch a row of plants. During the experiment, at 5:00 pm everyday, each plant was brushed 60 times within a minute at a constant speed of 0.4 m s^{-1} (rotating frequency of machine is 52 Hz). The mild speed can also relieve the touch damage of printing paper to plants. After each daily brushing cycle, the pots were turned 45 degree clockwise to ensure that MS was equally applied from different directions. This method had two advantages important to our study. First, it simulates the MS effect of wind without the concomitant effect on plant microclimate (temperature, evaporation and CO_2 concentration). Second, this setup allowed us to flex many plants simultaneously at the same speed, time and force, while preventing the physical damage that often occurs when plants are manually flexed (e.g. Niklas, 1998; Anten *et al.*, 2005; Anten *et al.*, 2009).

Each plant was supplied with 200 ml of water once every two days and 20 ml 0.1% nutrient solution (made of fertilizer powder; 20% N, including 3.94% ammonium nitrogen, 6.05% nitrate nitrogen and 10% urea nitrogen, 20% P, 20% K, 0.05% Mg, and trace elements; Peters Professional, Scotts, Marysville, Ohio, USA) twice during the experiment.

Measurements

On 29 July 2009, plant height was measured to determine the burial depth. After burial and defoliation, basal diameter of the stem was measured with a digital vernier caliper. The stem base of buried plants (the part just above the soil, D_B hereafter) was, therefore, on a relatively higher and younger part of the stem than in the other treatments (D_{NB}). For control and brushing treatments, we therefore also measured the diameter of the stem part (intermediate diameter, $D_{NB,int}$ hereafter) that was of the same age as the basal section (D_B) of the buried plants. On the same day seven seedlings were harvested and separated into leaves, stems, tubers and roots to determine initial biomass. Leaf images were obtained with a scanner (Uniscan e53, Qing Hua Ziguang, Beijing, China), and then leaf area was measured with ImageJ

(1.32j, National Institutes of Health, Bethesda, Maryland, USA). Subsequently all plant parts were dried at 70 °C for 48 h and dry mass was measured.

On 15 September 2009, all experimental plants were harvested. Plant height, basal diameter and fresh weight were measured. For control and brushing treatments, we also measured the intermediate diameter. The Young's Modulus (E), breaking stress (σ_b), the maximum load force (F_{load}) of the second stem internode above the soil were determined with the universal electromechanical testing machine (Type 5540, Instron, Norwood, Massachusetts, USA) using the approach described in Anten *et al.* (2005). In short, stem samples were fixed with two small clamps and a vertical force was applied on the stem halfway between these clamps. The distance between clamps fixing the stems was 5 cm. As such the aspect ratio (length / diameter) of all samples was larger than 20. Leaf area and dry biomass were measured using the methods as described above.

Data analysis

Because the burial treatments significantly changed the aboveground height and basal diameter of the plants, we calculated the relative rates of growth rather than the absolute ones.

Relative growth rate in terms of biomass increment (RGR_m ; $\text{g g}^{-1} \text{day}^{-1}$) was calculated as:

$$RGR_m = \frac{\ln W_2 - \ln(W_1 - W_{loss})}{T} \quad (1)$$

where T is the period (days) between the two harvests. W_{loss} , W_1 and W_2 are the biomass loss, initial total biomass and final total biomass, respectively. Here we define the leaf loss as the leaves that could not photosynthesize, including the clipped and buried leaves. In the final harvest, we did not find any leaves underground. As a result, we assume that the leaves under burial died very soon and were treated as lost.

Relative growth rate in terms of height increment (RGR_h ; $\text{cm cm}^{-1} \text{day}^{-1}$) was calculated as:

$$RGR_h = \frac{\ln H_2 - \ln H_1}{T} \quad (2)$$

where H_1 and H_2 are initial and final height, respectively. For burial treatment, height was measured from the burial surface to the top.

Relative growth rate of diameter (RGR_d ; $\text{mm mm}^{-1} \text{day}^{-1}$) was calculated as:

$$RGR_d = \frac{\ln D_2 - \ln D_1}{T} \quad (3)$$

where D_1 and D_2 are initial and final basal diameter, respectively.

The leaf area ratio (LAR; $\text{m}^2 \text{g}^{-1}$) and net assimilation rate (NAR; $\text{g m}^{-2} \text{day}^{-1}$) were calculated by the classical method (Anten & Ackerly, 2001):

$$LAR = \frac{A_2 / W_2 + (A_1 - A_{loss}) / W_1}{2} \quad (4)$$

$$NAR = \frac{W_2 - (W_1 - W_{loss})}{T} \times \frac{\ln(A_2) - \ln(A_1 - A_{loss})}{A_2 - (A_1 - A_{loss})} \quad (5)$$

where A_{loss} , A_1 and A_2 are the leaf area loss, initial and final leaf area, respectively.

The fraction of newly assimilated biomass allocated to the production of lamina tissue (f_{lam} ; g g^{-1}) was also estimated with the classical approach (Anten & Ackerly, 2001):

$$f_{lam} = \frac{L_2 + L_{loss} - L_1}{W_2 + W_{loss} - W_1} \quad (6)$$

where L_{loss} , L_1 and L_2 are the leaf mass loss, initial and final leaf mass, respectively.

The second moment of area (I ; m^4) describes the geometric contribution to rigidity of the material (Jaffe *et al.*, 1984; Niklas, 1996). It was calculated, assuming the stem to have circular shape, as:

$$I = \frac{\pi r^4}{4} \quad (7)$$

where r is the radius measured at the base of the stem for all plants (i.e., $D_B / 2$)

and $D_{NB} / 2$ for burial and non-burial plants, see also section *Measurements*, part on diameters).

The Young's modulus (E) was calculated from the slope of the vertical load force (F_{load}) displacement (δ) curve generated by the universal electromechanical testing machine as:

$$E = \frac{F_{load}L^3}{192\delta I_b} \quad (8)$$

where L is the length between the clamps. The radius used for calculation of I_b is very close to the point where the stem breaks. Maximum load force and displacement were recorded automatically by the machine. Flexural stiffness (EI ; $N\ m^2$) also describes the rigidity of the stem base and is the product of E and I , i.e., $EI = E * I$.

The breaking stress (σ_b), measured from the F_{load} exerted to the point where the stem broke, was calculated as:

$$\sigma_b = \frac{F_{load}Lr_b}{8I_b} \quad (9)$$

The buckling safety factor (BSF) that indicates the ability of plants to carry their own weight was calculated from the critical buckling height (H_c) and the real height (H_{real} ; Niklas, 1992), i.e.,

$$BSF = \frac{H_c}{H_{real}} \quad (10)$$

where H_c is the height beyond which the stem will deflect as a result of the plant's own weight and was calculated as:

$$H_c = \left(\frac{8EI}{P}\right)^{0.5} \quad (11)$$

where P is the fresh weight (N). This formula treats stems as idealized columns, ignoring tapering and uneven loading but the results tend to be

comparable to those of more complicated models (Holbrook & Putz, 1989; Moulia & Fournier-Djimbé, 1997; Henry & Thomas, 2002; Jaouen *et al.*, 2007). This is probably because the weight of leaves more or less compensates for stem tapering. In general, the use of simplified mechanical models such as eqn. 11 is sufficient for the qualitative comparisons of mechanical stability between plants of the same species with a very similar basic structure, as was done here.

The maximum lateral wind force ($F_{lateral}$) that plants resist before rupture occurs at the stem base was calculated as (Anten *et al.*, 2005):

$$F_{lateral} = \frac{\sigma_b \pi r^3}{4H} \quad (12)$$

where H is the median real height.

A three-way ANOVA was used to investigate the effects of simulated brushing, burial and defoliation, all of which were fixed factors. A two-way ANOVA was used to test the effects of position and brushing on diameter growth. The same method was used to test for the effects of burial and brushing on intermediate diameter growth. Before analysis, data were checked for equality of variance with Levene's test and for normality with Shapiro-Wilk test. SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA) was employed for the analyses. Ln-transformation was used on NAR, LAR and mechanical properties. Here we chose $P < 0.05$ as significance level.

Results

Mechanical properties

The effects of brushing (MS) on the mechanical stem traits depended strongly on whether plants were partially buried or not. Basal diameter growth (RGR_d) was enhanced by brushing (Table 1, Fig. 1a), resulting in a larger second moment of area of stems (I , eqn. 7; Table 2, Fig. 2b) in buried plants, but this pattern was not observed in unburied ones. Similarly the Young's modulus (E) was increased by MS in buried plants but not in unburied ones (Table 2, Fig. 2a). The breaking stress (σ_b) was increased by brushing in both burial and un-burial treatments (Table 2, Fig. 2g). Burial alone also stimulated relative diameter growth rate (Table 1, Fig. 1a), but the value of final basal diameter was still smaller than that of the control plants (Fig. 1b), resulting in a significantly lower I (Table 2, Fig. 2b). This can be explained from the fact

that in the buried plants the diameter of the stem section just above soil was inevitably on a higher part of the plant, and due to stem tapering thus thinner. Defoliation by contrast did not have any significant effect on RGR_d , E or I (Table 1, 2, Fig 1a, 2a, b).

Table 1. Results of ANOVA for the effects of brushing, burial, defoliation and their interactions on biomass allocation and growth traits of *Cynanchum komarovii*.

Growth trait	Brushing (Br)	Burial (Bu)	Defoliation (De)	Br×Bu	Br×De	Bu×De	Br×Bu×De
Biomass allocation to leaves	0.02 ^{ns}	9.09 ^{**}	10.79 ^{**}	0.63 ^{ns}	1.07 ^{ns}	0.24 ^{ns}	1.38 ^{ns}
Biomass allocation to roots	0.57 ^{ns}	0.04 ^{ns}	4.69 [*]	1.00 ^{ns}	1.39 ^{ns}	0.70 ^{ns}	1.06 ^{ns}
Biomass allocation to stems	0.23 ^{ns}	0.04 ^{ns}	8.31 [*]	2.12 ^{ns}	1.08 ^{ns}	0.17 ^{ns}	0.07 ^{ns}
Biomass allocation to tubers	0.12 ^{ns}	0.79 ^{ns}	3.29 ^{ns}	0.00 ^{ns}	0.69 ^{ns}	0.47 ^{ns}	0.36 ^{ns}
Leaf to stem ratio	0.15 ^{ns}	18.18 ^{***}	68.01 ^{***}	2.53 ^{ns}	5.07 [*]	1.32 ^{ns}	1.62 ^{ns}
f_{lam}	2.81 ^{ns}	0.70 ^{ns}	0.13 ^{ns}	2.24 ^{ns}	0.73 ^{ns}	0.44 ^{ns}	0.04 ^{ns}
RGR_m	18.29 ^{***}	2.86 ^{ns}	25.04 ^{***}	1.18 ^{ns}	0.66 ^{ns}	11.48 ^{**}	4.59 [*]
RGR_d of basal	9.40 ^{**}	50.69 ^{***}	2.47 ^{ns}	17.71 ^{***}	0.03 ^{ns}	3.06 ^{ns}	0.01 ^{ns}
RGR_h	2.59 ^{ns}	36.31 ^{***}	0.12 ^{ns}	0.35 ^{ns}	0.80 ^{ns}	2.87 ^{ns}	0.28 ^{ns}
Basal diameter	4.71 [*]	40.69 ^{***}	1.18 ^{ns}	10.06 ^{**}	0.08 ^{ns}	2.26 ^{ns}	0.00 ^{ns}
Height	3.10 ^{ns}	23.37 ^{***}	0.01 ^{ns}	0.24 ^{ns}	0.96 ^{ns}	0.97 ^{ns}	0.03 ^{ns}
LAR	0.97 ^{ns}	84.23 ^{***}	98.29 ^{***}	2.37 ^{ns}	6.70 [*]	0.14 ^{ns}	1.20 ^{ns}
NAR	14.31 ^{***}	0.55 ^{ns}	11.21 ^{**}	0.09 ^{ns}	1.26 ^{ns}	10.85 ^{**}	6.39 [*]

F values and the significance levels (^{***} $P < 0.001$, ^{**} $P < 0.01$, ^{*} $P < 0.05$ and ^{ns} $P \geq 0.05$) are given; degree of freedom from all the effects is (1, 48); data were Ln-transformed before analyses.

A direct consequence of partial burial is that the basal stem part (the part just above the soil) is on a younger internode situated more towards the tip of the plant. Thus among unburied plants we tested whether RGR_d and the effects of MS there upon depended on position on the stem. This was done by also measuring diameters of stem section ($D_{NB,int}$) on unburied plants that were developmentally the same as the basal stem section of buried plants. This showed that for unburied plants basal stem sections exhibited slower RGR_d values than intermediate stem sections (D_{NB} vs. $D_{NB,int}$; $P = 0.024$). Brushing

however did not significantly affect RGR_d of either stem section ($P = 0.146$).

The brushing by burial interaction on stem mechanical traits was reflected in similarly interactive effects of these factors on the characteristics that indicate whole-stem mechanical behavior. The buckling safety factor (BSF), maximum lateral force ($F_{lateral}$), as well as the stem flexural stiffness (EI) were all increased by brushing in buried plants but not in unburied plants (Table 2, Fig. 2c, e, g). Brushing similarly increased the H_c only in the buried plants (Table 2, Fig 2f), without significantly affecting the real height of plants (H_{real} ; Table 2, Fig. 1d). Defoliation enhanced the H_c and increased the BSF (Table 2, Fig. 2f, g).

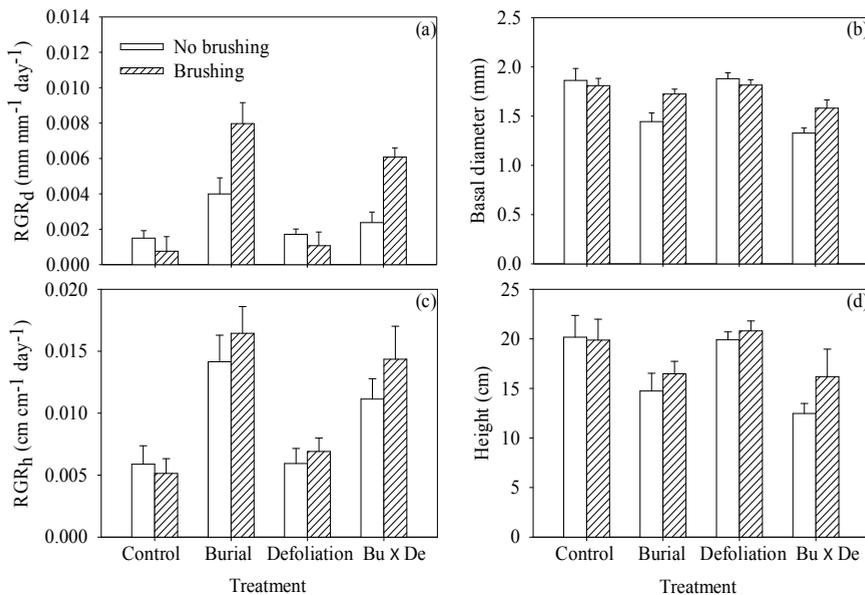


Fig. 1. Relative basal diameter growth rate (a), basal diameter (b), relative height growth rate (c) and height (d) of *Cynanchum komarovii* subjected to brushing, burial and defoliation treatments. Data are mean \pm SE without transformation.

Growth responses

Both burial and defoliation reduced the leaf mass fraction because of the initial leaf loss (Table 1). Burial and defoliation also decreased LAR (Table 1, Fig. 3a) and leaf to stem ratio (Table 1, Fig. 3d). Brushing increased RGR_m of *C. komarovii* but defoliation decreased it (Table 1, Fig. 3c). Burial boosted

both basal diameter and height growth but the other two factors did not (Table 1, Fig. 1a, c). Except for the combination of burial and brushing treatment, NAR of the brushed plants was higher than the non-brushed ones (Table 1, Fig. 3b). None of the three factors had effect on the fraction of newly assimilated biomass allocated to leaves (f_{lam} ; Table 1). The combination of burial and defoliation resulted in a reduction in both NAR and RGR_m (Table 1, Fig. 3b, c). Among defoliated plants, brushing increased leaf to stem ratio (Table 1, Fig. 3d).

Table 2. Results of ANOVA for the effects of brushing, burial, defoliation and their interactions on mechanical properties of *Cynanchum komarovii*.

Mechanical property	Brushing (Br)	Burial (Bu)	Defoliation (De)	Br×Bu	Br×De	Bu×De	Br×Bu×De
Young's Modulus	0.00 ^{ns}	0.11 ^{ns}	0.25 ^{ns}	6.22*	0.15 ^{ns}	3.16 ^{ns}	0.11 ^{ns}
Second moment of area	2.27 ^{ns}	34.65 ^{***}	0.85 ^{ns}	7.33**	0.09 ^{ns}	1.44 ^{ns}	0.00 ^{ns}
Flexural stiffness	0.94 ^{ns}	18.51 ^{***}	0.11 ^{ns}	7.34**	0.00 ^{ns}	0.09 ^{ns}	0.00 ^{ns}
Breaking stress	16.98 ^{***}	23.51 ^{***}	0.03 ^{ns}	4.01 ^{ns}	2.02 ^{ns}	3.13 ^{ns}	2.03 ^{ns}
Maximum lateral force	10.79**	22.85 ^{***}	0.52 ^{ns}	4.21*	1.66 ^{ns}	2.50 ^{ns}	1.56 ^{ns}
Critical buckling height	0.12 ^{ns}	12.53**	8.04**	16.54 ^{***}	1.69 ^{ns}	0.06 ^{ns}	0.14 ^{ns}
Buckling safety factor	0.07 ^{ns}	1.00 ^{ns}	4.30*	3.29 ^{ns}	1.22 ^{ns}	1.58 ^{ns}	0.18 ^{ns}

F values and the significance levels (^{***} $P < 0.001$, ^{**} $P < 0.01$, ^{*} $P < 0.05$ and ^{ns} $P \geq 0.05$) are given; degree of freedom from all the effects is (1, 48); data were Ln-transformed before analyses.

Discussion

Sand burial modifies the effects of MS on mechanical traits

The effects of MS on stem and whole-plant mechanical traits depended clearly on the presence or absence of sand burial. Among buried plants, MS induced a considerable increase in stem diameter growth (RGR_d), slightly increased the

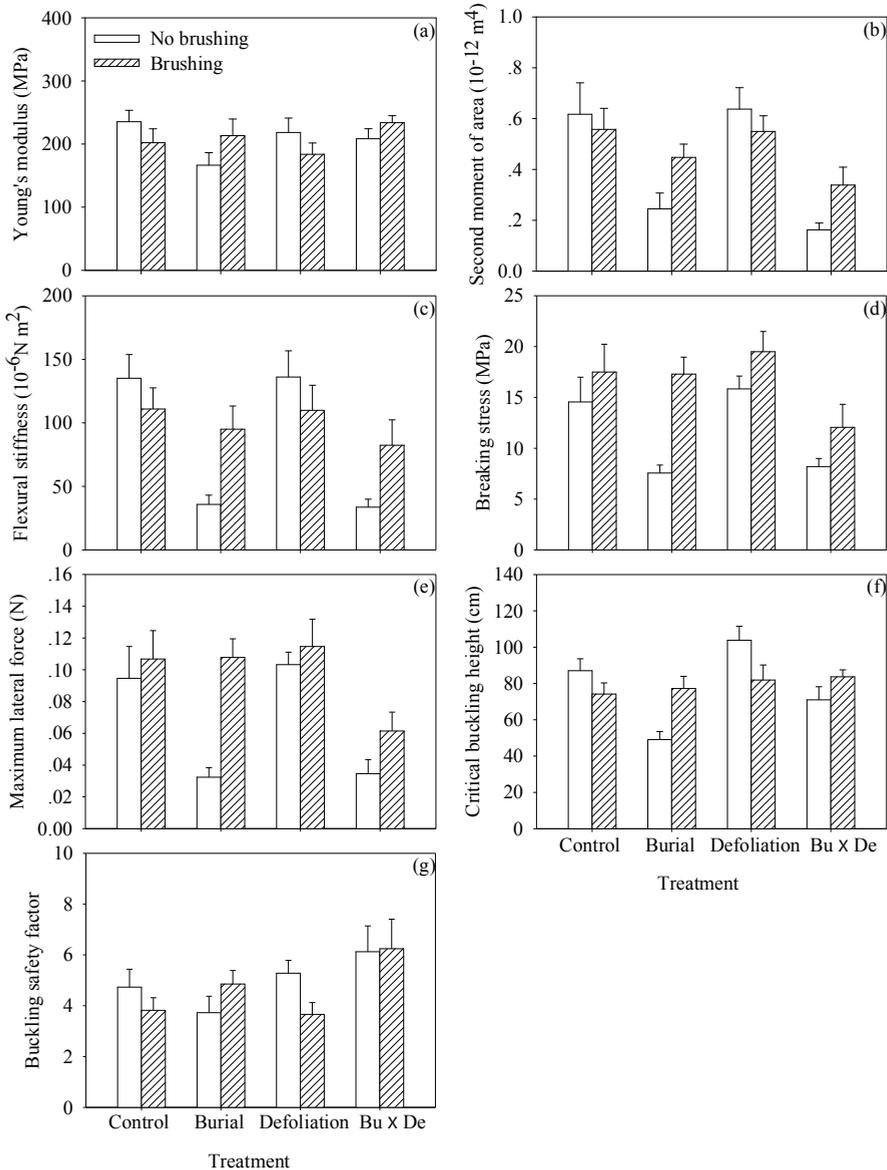


Fig. 2. Young's modulus (a), second moment of area (b), flexural stiffness (c), breaking stress (d), maximum lateral force (e), critical buckling height (f) and buckling safety factor (g) of *Cynanchum komarovii* subjected to brushing, burial and defoliation treatments. Data are means \pm SE without transformation

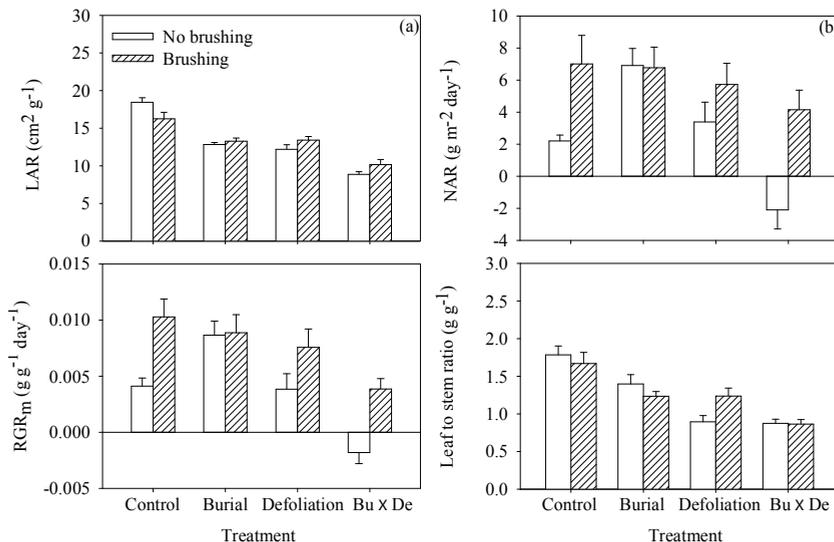


Fig. 3. Leaf area ratio (a), net assimilation rate (b), relative growth rate (c) and leaf to stem ratio (d) of *Cynanchum komarovii* subjected to brushing, burial and defoliation treatments. Data are mean \pm SE without transformation

Young's modulus (E) and led to a more than two-fold larger flexural stiffness (EI). Consequently stems of brushed plants were calculated to resist larger lateral force ($F_{lateral}$) than stems of unbrushed plants. Interestingly however this pattern was not observed among unburied plants. Brushing had no significant effects on E , EI or $F_{lateral}$, which indicated that plant responses to MS may depend on the level of sand burial. Similar interactive effects on stem mechanical traits were also observed in a separate experiment on *Caragana intermedia* in 2010 (L. Xu unpublished data). As far as we know, we are the first to demonstrate this interactive effect. In drylands, strong wind can cause sand movement, and MS and sand burial may often occur simultaneously. Their interactive effects on plant traits may thus play an important role in shaping plant structure in these environments. In addition to the MS by sand burial interaction shown by us, other studies showed that MS effects on plant traits can interact with those of water (Wang *et al.*, 2008; Wang *et al.*, 2009b), shading (Anten *et al.*, 2005; Anten *et al.*, 2009) or nutrients (Grace *et al.*, 1982). Together these results indicate that the functional significance of thigmomorphogenesis under natural field conditions cannot be viewed independently from other stress factors that are present.

The question remains what mechanisms might be responsible for the fact that MS effects were significant in buried but not unburied plants. Mechanical

architecture is not only related with growth forms of plants, but also with different growth stage (Isnard *et al.*, 2003). Differential responses to MS might have been associated with the fact that as burial raises the soil level, the basal stem section (i.e, the part being closest to the soil) will inevitably be younger in a buried than an unburied plant. The difference in response to MS between buried and unburied plants could thus be associated with the fact that younger tissue tends to exhibit stronger thigmomorphogenetical responses to stress than older one (Biddington, 1986), and is generally more plastic in its responses to external stimuli (Chehab *et al.*, 2009). It has also been reported that E decreases from young to old stems in ontogeny (Isnard *et al.*, 2003). However, in the unburied plants, RGR_d of younger intermediate stem sections was not significantly affected by brushing, suggesting that tissue age can not fully explain the MS by burial interactive effects observed in this study.

Another explanation could be that the mechanical effect of brushing differed between the burial treatments. It has been suggested that diameter growth regulation by MS may depend on the strength of the local mechanical signal (Coutand *et al.*, 2009). A direct consequence of burial was that at the beginning of the experiment plant height above the soil was twice as large in the unburied plants (14.9 ± 0.27 cm) as in the buried ones (7.5 ± 0.46 cm), while the basal diameter was 30% larger, i.e., 1.75 ± 0.04 mm and 1.17 ± 0.02 mm in the unburied and buried, respectively. Brushing was applied to the tips of plants at a constant speed (0.4 m/s). Since longer stems require less force to bend and thinner stems experience more stress when subjected to a given bending moment (Gere & Timoshenko, 1999), stress at the base of the stem was probably larger in the buried than in the unburied plants, which in turn, may have induced a stronger thigmomorphogenic response.

The contention above would have to be tested in more detail, using e.g. local stress measurements. It is also possible that some other unknown factor associated with burial might have been responsible for the differences in MS effects between buried and unburied plants. More research is thus needed to understand why partially buried plants respond differently to MS than unburied ones.

The functional significance of the MS response in buried plants might be associated with the fact that burial resulted in a more than two-fold increase in the relative stem elongation rate (RGR_h). Furthermore, there was a greater absolute stem elongation response in buried plants than unburied ones (29%). As a result the initial 50% difference in height above the soil was reduced to a difference of only 27%. Increased stem elongation in response to burial has been reported in several other studies (Brown, 1997; Dech & Maun, 2006) and

is believed to be an important response that enables plants to tolerate burial. But the potential consequences of this burial-induced stem elongation for mechanical stability of plants have not been considered. Stem elongation may entail an increased mechanical risk if there is no concomitant increase in stem diameter, strength or rigidity of stem tissue (Niklas, 1992). In the Mu Us Sandland, plants are exposed to highly unpredictable wind which varies both in time and space. Our data suggest that low levels of MS (i.e., a brushing treatment that simulates 0.4 m s^{-1} wind speed) serve as a cue for the adjustment of allometric growth patterns in order to avoid future mechanical damage. In this context it is worth noting that when our plants were not exposed to MS, burial ultimately resulted in a three fold reduction in the $F_{lateral}$ that plants can resist. By contrast among brushing plants this difference in strength was negligible. Consistent with those studies, others (Niklas, 1999; Anten *et al.*, 2009) observed that in the absence of MS (e.g. in greenhouse experiments) plants can exceed their critical buckling height.

Contrary to burial, defoliation had no effect on the mechanical properties, neither the interaction with brushing. But it increased the buckling safety factor (BSF) in terms of a greater critical height as the leaf removal reduced the static load (fresh weight) of the plant at the same actual height.

MS, burial and defoliation effects on growth

In the defoliation treatment half of the leaves were removed and in the burial treatment about a third of the leaves were covered with sand. Both treatments thus greatly reduced the photosynthetically active area of the *C. komarovii* plants, and burial in addition could also reduce O_2 availability to roots. Defoliation itself had a negative effect on growth, but burial itself had no significant effect. However there was a clear interactive effect of the two factors (see the significant $\text{Bu} \times \text{De}$ interaction, Table 1) with burial strongly aggravating the negative growth effects of defoliation. This can probably be explained with the limiting resource model (LRM; Wise & Abrahamson, 2007), which proposes that plant tolerance to herbivory depends on the type of resource and herbivore under consideration. If the herbivory-induced damage to the plant affects the acquisition of the most limiting resource, tolerance to damage will be low, e.g. defoliation affecting light acquisition under shaded conditions (Anten *et al.*, 2003). In view of that, the effects of defoliation should be expected to be aggravated by burial, as the latter entails further reduction in light capture. In many arid or semi-arid regions, sand movement and the resulting burial of plants are primarily due to overgrazing-induced desertification. Our findings indicate that burial then further aggravates the

effects of overgrazing, thus creating a positive feedback on this process. This should be taken into account when considering the sustainability of grazing and the potential for vegetation collapse in dry regions.

Brushing alone increased RGR_m and resulted in a faster growth in *C. komarovii*, agreeing with the conclusions of a previous study (Mitchell, 1996) that low-amplitude mechanical vibration, whose frequency ranges from 50 to 60 Hz, promotes rather than inhibits cumulative plant growth. However, many studies found that MS could negatively affect plant performance, i.e. reduce biomass (Niklas, 1998; Wang *et al.*, 2008; Anten *et al.*, 2010), reproduction (Niklas, 1998; Cipollini, 1999) and/or stem elongation (Neel & Harris, 1971). Coutand & Moulia (2000) reported the cessation of growth a few minutes after bending. Recently, Liu *et al.* (2007b) showed that MS did not affect either RGR_m or the final standing mass of *Potentilla reptans* at the whole plant level. The different results for the effects of MS on growth could be due to the differences in the type and intensity of MS applied. In our case as in Mitchell (1996) the level of MS was relatively low; our treatment simulated the mechanical effect associated with a 0.4 m s^{-1} wind speed. Plants under brushing had a higher net assimilation rate (NAR) than the unbrushed ones, which partially contributed to the high RGR_m . On the other hand, the fractional allocation of biomass was not affected by brushing, as also reported in other studies (Ashby *et al.*, 1979; Biddington & Dearman, 1985; Gartner, 1994; Wang *et al.*, 2009b).

Our study is the first to show that the effects of mechanical stress on plants can depend on the level of sand burial. In addition we show that burial may also aggravate the negative effects of defoliation on growth. In dry sandlands MS, burial and defoliation typically occur simultaneously and their interactive effects should be taken into account in investigation on plant performance and associated vegetation processes in these areas. More work is also needed to understand the physiological mechanisms that underlie the interactive effect of burial and MS.

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Greenhouse in the field station

Chapter 3

Trampling, defoliation and physiological integration affect growth, morphological and mechanical properties of a root-suckering clonal tree

with Fei-Hai Yu, Elles van Drunen, Feike Schieving, Ming Dong and Niels P.R. Anten

Abstract

- Grazing is a complex process involving both trampling and defoliation. Shifts in allocation from roots to shoots following defoliation could increase vulnerability to trampling. Though physiological integration has been proved to facilitate tolerance to many stress factors, its interactive effect on grazing have not been explicitly studied.
- In a field study, small and large ramets (individuals) of a root-suckering clonal tree *Populus simonii* were subjected to trampling, defoliation and disconnection to other ramets. We measured survival, growth, morphological and stem mechanical traits.
- Disconnection and trampling increased mortality, especially in small ramets. Trampling increased stem length, basal diameter, fibrous root mass, stem stiffness and resistance to deflection in connected ramets, but decreased them in disconnected ones. Trampling decreased vertical height more in disconnected than in connected ramets, and reduced stem mass in disconnected ramets but not in connected ones. Defoliation reduced basal diameter, leaf mass, stem mass and leaf area ratio, but did not interact with trampling or disconnection.
- Although clonal integration did not influence defoliation response, it did alleviate the effects of trampling. We suggest that by facilitating resource transport between ramets, clonal integration compensates for damage induced by trampling to fine roots.

Key words: Biomechanics, clonal integration, drylands, grazing, *Populus simonii*, resource sharing, root connections, root severing.

Introduction

Grassland degradation and desertification is a big problem in drylands (Squires, 2003; Verón & Paruelo, 2010). Overgrazing is argued to be the major cause of desertification worldwide, though other factors also play a role (Yoav *et al.*, 2006). Grazing is a complex process that has several effects on plants, and the most important ones are biomass consumption and associated defoliation (Hanley & Sykes, 2009; Gruntman & Novoplansky, 2011), trampling and associated mechanical stress, and changes in soil properties such as compaction, nutrient addition (Sørensen *et al.*, 2009) and availability of soil silicon (Garbuzov *et al.*, 2011).

Trampling can strongly affect plant performance, community structure and species composition (Cole, 1988; Sun & Liddle, 1993a; Kobayashi *et al.*, 1997; Striker *et al.*, 2011), and in drylands it has been implicated as a cause of desertification (Ibáñez *et al.*, 2007). Trampling has a direct mechanical effect on plants by causing physical damage through either excess flexural loading or crushing plant organs (Sun & Liddle, 1993a). However, compared with other forms of mechanical stress such as wind loading and manual flexing and rubbing (known as “thigmomorphogenesis”), trampling acts on one point instead of on the whole plant and entails a discontinuous overwhelming impact, especially for small individuals. Several mechanical traits, including leaf toughness, root strength and stem flexibility, have been suggested to play a role in plant tolerance to trampling (Kobayashi *et al.*, 1999; Striker *et al.*, 2011). Most studies on trampling, however, were conducted at the community level (Cole, 1988; Cole, 1995; Servane & Francoise, 2003; Andrés-Abellán *et al.*, 2006), and few focused on the plastic responses of individual plants in terms of e.g. biomass allocation and mechanical traits. Sun & Liddle (1993a) argued that short plants may be more resistant to trampling because they tended to be protected by taller plants. In moving or semi-moving dunes of drylands where vegetation is sparse, studying responses of individual plants to trampling is more practical. While plastic responses of plants to other forms of mechanical stress have been well documented, little work has been done on trampling.

Biomass consumption and associated defoliation cause both direct loss of resources and reductions in photosynthetic surface. Yet plants exhibit compensatory traits that enable them to mitigate the negative effects of defoliation (Gruntman & Novoplansky, 2011; Muola *et al.*, 2011; Zhang *et al.*, 2011). The capacity for compensatory growth in turn may depend on the presence of other factors. It is found that dry or nutrient-poor conditions favor

compensatory growth, whereas shading tends to reduce it (Coughenour *et al.*, 1990; Anten *et al.*, 2003; Wise & Abrahamson, 2007). While defoliation and trampling occur simultaneously during grazing, their potential interactive effects on plants have not been investigated. Plants respond to defoliation typically by increasing allocation to leaves at the expense of reduced allocation to roots and stems (Stevens *et al.*, 2008), which could make plants more vulnerable to mechanical damage through trampling.

Clonal growth has been considered as an important mechanism to allow plants to persist in adverse conditions (Beatty & Provan, 2011). Many plants in drylands exhibit clonal growth patterns whereby ramets are produced along horizontal runners (e.g., rhizomes, stolons or roots; Yu *et al.*, 2008; Gruntman & Novoplansky, 2011; Sui *et al.*, 2011). Connections between individuals (ramets) usually persist for several years, allowing the exchange of resources such as carbohydrates, water and nutrients (Yu *et al.*, 2004; Yu *et al.*, 2008; Roiloa *et al.*, 2007; de Witte & Stöcklin, 2010). This physiological integration has been shown to enhance the performance of ramets suffering from different local stresses, including shading (Alpert, 1999; Alpert *et al.*, 2003), water shortages (de Kroon *et al.*, 1996; Dong & Alaten, 1999), nutrient depletion (Alpert, 1991), burial (Yu *et al.*, 2004) and erosion (Yu *et al.*, 2008). However, no study has examined the role of clonal integration in determining plant tolerance to trampling.

We conducted a field experiment, in which ramets of the common local tree species *Populus simonii* were subjected to two levels of root-severing (the horizontally growing roots connected to the ramets were disconnected or not), defoliation and trampling. Specifically, we address the following questions: (1) What are the effects of trampling and defoliation on growth and on morphological and mechanical traits, and are these effects additive or interactive? (2) To what extent are trampling and defoliation effects changed by physiological integration, i.e., the presence of clonal connections between ramets?

Material and Methods

Study site and focal species

The experiment was conducted in the mobile dunes near Ordos Sandland Ecological Research Station (OSES, 39°29'37.6"N, 110°11'29.4"E, 1300 m a.s.l.) of the Institute of Botany, the Chinese Academy of Science, located in Mu Us Sandland in Inner Mongolia, China. The average summer temperature is 20–24 °C, and average annual precipitation is 260–450 mm, concentrating

from July to September (Zhang, 1994). Due to severe desertification in the past decades, this area, which used to be typical grassland, is now dominated by different types of sandland, including fixed, semi-mobile and mobile dunes (Zhang, 1994). This desertification is due to the persistent overgrazing in this area (Huang *et al.*, 2000). The mobile dunes where the experiment was conducted are mainly occupied by *Populus simonii*. There are also a few plants of other species, including *Artemisia ordosica*, *Caragana intermedia*, *Cynanchum komarovii* and *Hedysarum laeve*.

Populus simonii Carr. (Salicaceae) is a tree that can reach a height of 20 m and a stem diameter of 0.5 m (Wang & Fang, 1984). As it is highly resistant to cold, drought and wind, it is widely distributed in many provinces in north and southwest China. *P. simonii* is a root-suckering clonal species whose roots can extend horizontally; new ramets are produced on these roots. The horizontal roots in the study area mainly occur within the top 0.1 m of sand (L. Xu personal observations). The small ramets from horizontal roots are often grazed by animals. The horizontal roots can generate small fibrous roots. In mobile dunes, the roots are easily damaged by exposure to the air when they are denuded, or by trampling. *P. simonii* is an important local species used for the stabilization of mobile dunes and recovery of vegetation in many parts of northern China (Liu & Man, 2008).

Experimental design

On 5 June 2010, we selected 115 large ramets and 115 small ramets of *P. simonii* on three adjacent mobile dunes within an area of about 0.32 km². At the start of the experiment, average ramet height and stem basal diameter were 26.65 ± 0.55 cm and 6.70 ± 0.23 mm, respectively, for the large ramets, and 11.77 ± 0.35 cm and 4.30 ± 0.14 mm for the small ones. Of the 115 ramets in each size class, 15 were randomly selected and harvested to measure initial biomass. Immediately after harvesting, these plants were enclosed in polyethylene bags and taken back to the field station. The ramets were divided into leaves, stems, main roots and fibrous roots. Leaf images were obtained with a scanner (Uniscan e53, Qing Hua Ziguang, Beijing, China), and leaf area was measured with ImageJ (1.32j, National Institutes of Health, Bethesda, Maryland, USA). Then all plant parts were dried at 70 °C for 48 h and dry mass was measured.

The remaining 100 ramets were randomly subjected to two levels of trampling treatments (trampling *vs.* no trampling), two levels of defoliation treatments (defoliation *vs.* no defoliation) and two levels of root-severing treatments (severing *vs.* no severing, *i.e.*, the horizontal roots that connected

the ramets were severed or not to prevent or allow clonal integration) in a factorial design. There were 15 replicates for the four treatments involving root-severing and 10 for the other four treatments without root-severing because we expected that in the former the ramets would suffer from a higher mortality.

Trampling was applied using a manually constructed load with weight (6.22 kg) and surface area (diameter 3.07 cm) such that, when placed on a plant, it produced a stress level (0.84 kg/cm^2) similar to that produced by the hoof of a sheep weighing 40 kg (Lin *et al.*, 2008). Trampling was simulated by placing the load vertically for 3 seconds on the base of the stem. Every time we trampled the plants three times in different directions. We did trampling twice a week from 8 June until the plants were harvested. For the defoliation treatment, 50% of the leaves were removed by clipping one of every two leaves along the stems, top to bottom. For the severing treatments, we carefully removed the sand to the level at which the horizontally growing roots connecting the ramets were exposed. We cut off the horizontal roots 5 cm away from both sides of the ramet with sharp scissors and then put the sand back. For non-severing treatments, we also exposed the horizontally growing roots connected to the ramets and then reburied them to avoid the potential confounding effects of disturbance. During these processes, care was taken not to disturb the adventitious roots of the ramets. During the experiment the defoliation and root-severing treatments were done only once (on June 6) and the trampling treatments were done twice a week.

From 11 September 2010 to 14 September 2010, we harvested all the surviving plants. Length, deflection angle from vertical, and basal diameter were measured in the field. Ramets were then excavated and transported to the lab of the field station. Leaves were scanned, leaf area was measured by ImageJ, and leaf mass obtained after drying at 70 °C for 48 h. We measured Young's modulus, breaking stress and maximum load force of the main stem of each surviving ramet, with a universal electromechanical testing machine (Type 5540, Instron, Norwood, Massachusetts, USA), applying the three-point bending technique (for details see Anten *et al.*, 2005). In short, stem samples were fixed at both ends in small clips and a vertical force was applied on the stem halfway between these clamps. We adjusted the distance between the clips to make sure it was at least ten times larger than the basal stem section. Dry mass of roots, stems and leaves were then measured using the methods described above.

Data processing

We calculated aboveground leaf area ratio (LAR; $\text{m}^2 \text{g}^{-1}$), the second moment of area (I ; m^4 , i.e., the geometric contribution to stiffness of the stem; Jaffe *et al.*, 1984; Niklas, 1996), Young's modulus (E , i.e, stiffness of an elastic material; Gere & Timoshenko, 1999), flexural stiffness (EI ; N m^2) of the rigidity of a stem cross section and the stem breaking stress (σ_b ; MPa) according to the equations in Anten *et al.* (2005) and Anten & Ackerly (2001).

The mechanical traits mentioned above were scaled up to calculate resistance to bending and breaking at the whole-stem level. We note that these are qualitative measures of stem mechanical behavior solely used for comparative purposes. Resistance to bending force (F_{bend}), referring to bend the stem by a small angle (θ), was calculated with following formula (Gere & Timoshenko, 1999):

$$F_{\text{bend}} = \frac{2EI\theta}{H_v^2} \quad (1)$$

where H_v is the vertical height of the main stem from the force acting point to the ground. Here we assume $\theta = 1^\circ$ because larger deflections require a different bending model (Gere & Timoshenko, 1999). We note that this is a qualitative measure of stem flexibility solely used for comparative purposes.

Vertical height (H_v) is the height between the sand and the highest tip of the main stem given by the following formula:

$$H_v = H * \sin \beta \quad (2)$$

where β is angle between main stem and ground.

The minimum lateral breaking force (F_{break}) exerting on the top of the stem, required to rupture the stem at its base, was calculated as (Gere & Timoshenko, 1999):

$$F_{\text{break}} = \frac{\pi d^3 \sigma_b}{32H_v} \quad (3)$$

where d is the basal diameter.

As noted in the introduction, trampling tends to entail an overwhelmingly large force and trampling can thus only break a favorable balance between

strength and flexibility and result in a low resistance to bending. Here we use the ratio of F_{break} to F_{bend} as a simple proxy for this balance:

$$\frac{F_{\text{break}}}{F_{\text{bend}}} = \frac{\sigma_b H_v}{Ed\theta} \quad (4)$$

As mentioned above we assume $\theta = 1^\circ$. The equation shows that the balance between strength and flexibility increases with the breaking stress (σ_b) and shoot vertical height (H_v) and decreases with Young's modulus (E) and basal diameter (d).

Statistical analysis

We used generalized linear model to analyze the effect of size, trampling, defoliation and severing on ramet survival, with logit as the link function. A three-way ANOVA was used to examine the effects of trampling, defoliation, severing and their interactions on growth, morphological and mechanical traits of the surviving, large ramets. All the data were ln-transformed and examined by Levene's test for equality of variance and the Shapiro-Wilk test for normality. We did not apply this analysis to the small ramets because 93% of the ramets in the root-severing treatment died. All analyses were conducted with SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA). The effects are considered significant if $P < 0.05$.

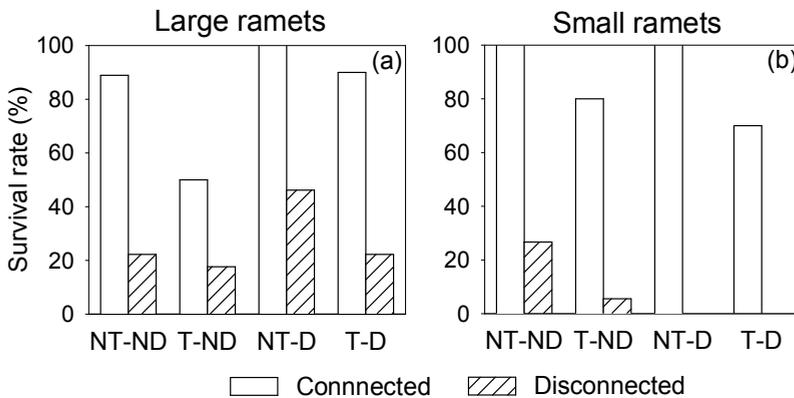


Fig. 1. Survival rate of large (a) and small (b) *Populus simonii* ramets subjected to root severing (disconnected) or not (connected), defoliation (D) or not (ND) and trampling (T) or not (NT).

Table 1. Effects of trampling, defoliation and severing clonal connections on the growth, morphological and mechanical properties of large ramets of *Populus simonii*.

	Trampling (T)	Defol. (D)	Severing (S)	T×S	D×S	D×T	D×T×S
Growth and morphological properties							
Main stem length	0.00 ^{ns}	1.17 ^{ns}	0.23 ^{ns}	5.77 [*]	0.09 ^{ns}	0.07 ^{ns}	0.34 ^{ns}
Vertical height	21.00 ^{***}	0.02 ^{ns}	0.04 ^{ns}	6.02 [*]	1.86 ^{ns}	1.56 ^{ns}	3.09 ^{ns}
Basal diameter	0.22 ^{ns}	8.66 ^{**}	7.01 [*]	7.82 ^{**}	0.05 ^{ns}	0.78 ^{ns}	0.38 ^{ns}
Leaf mass	4.49 ^{**}	5.49 ^{**}	32.75 ^{***}	0.72 ^{ns}	2.07 ^{ns}	1.32 ^{ns}	0.99 ^{ns}
Stem mass	1.88 ^{ns}	5.08 [*]	10.82 ^{**}	4.19 [*]	0.16 ^{ns}	0.16 ^{ns}	1.33 ^{ns}
Fibrous root mass	0.33 ^{ns}	0.07 ^{ns}	5.02 [*]	6.09 [*]	0.09 ^{ns}	1.92 ^{ns}	0.00 ^{ns}
Leaf area ratio	0.01 ^{ns}	22.69 ^{***}	2.94 ^{ns}	0.54 ^{ns}	0.01 ^{ns}	0.05 ^{ns}	0.14 ^{ns}
Mechanical properties							
Young's modulus	0.84 ^{ns}	0.88 ^{ns}	4.44 [*]	4.30 [*]	0.22 ^{ns}	0.01 ^{ns}	0.08 ^{ns}
Second moment of area	0.22 ^{ns}	8.66 ^{**}	7.01 [*]	7.82 [*]	0.05 ^{ns}	0.78 ^{ns}	0.38 ^{ns}
Flexural stiffness	0.98 ^{ns}	9.55 ^{**}	12.51 ^{**}	12.53 ^{**}	0.00 ^{ns}	0.58 ^{ns}	0.54 ^{ns}
Breaking stress	0.15 ^{ns}	0.01 ^{ns}	1.67 ^{ns}	5.06 [*]	0.16 ^{ns}	0.01 ^{ns}	1.68 ^{ns}
Breaking force (F_{break})	1.25 ^{ns}	4.19 [*]	12.48 ^{**}	12.95 ^{**}	0.02 ^{ns}	0.76 ^{ns}	2.51 ^{ns}
Bending force (F_{bend})	7.17 [*]	7.42 [*]	16.51 ^{***}	10.38 ^{**}	0.02 ^{ns}	1.05 ^{ns}	0.27 ^{ns}
$F_{\text{break}}/F_{\text{bend}}$	9.06 ^{**}	3.20 ^{ns}	3.79 ^{ns}	0.00 ^{ns}	0.11 ^{ns}	0.27 ^{ns}	2.03 ^{ns}

F values and the significance levels (^{***} $P < 0.001$, ^{**} $P < 0.01$, ^{*} $P < 0.05$ and ^{ns} $P \geq 0.05$) are given; degree of freedom of dry biomass of fibrous roots are (1,34); degree of freedom of the others from all the effects are (1,40); Data were Ln-transformed before analyses.

Results

Survival rate

Survival rate of the ramets was significantly affected by ramet size (Likelihood ratio $\chi^2 = 4.05$, $P = 0.042$), root-severing ($\chi^2 = 108.64$, $P < 0.001$) and trampling ($\chi^2 = 11.63$, $P = 0.001$), but not by defoliation ($\chi^2 = 0.37$, $P = 0.559$). The presence of root connections between ramets had a strong positive effect on survival, and this effect was larger for smaller than for larger plants

(Fig. 1). At the end of the experiment, survival rates of large connected ramets ranged from 50% to 100% and those of large disconnected ones from 18% to 46%. For the small ramets, survival rates were 30% to 100% for connected treatments and 0 to 27% for the disconnected ones.

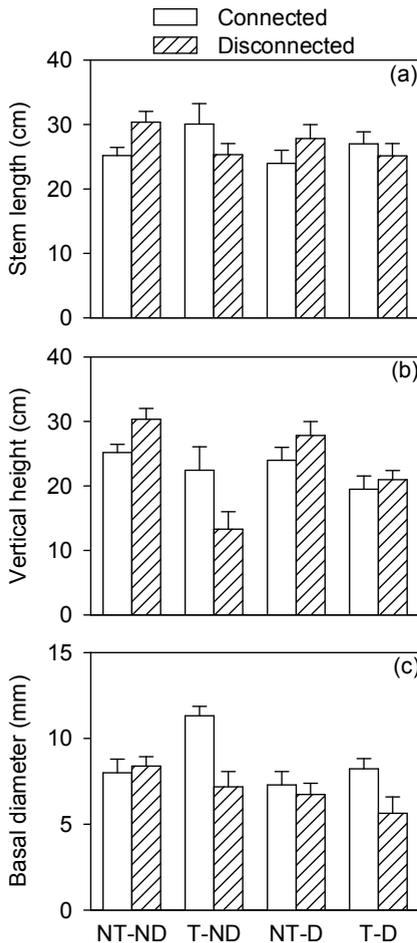


Fig. 2. Length of main stem (a), vertical height (b) and basal diameter (c) of the large *Populus simonii* ramets subjected to root severing (disconnected) or not (connected), defoliation (D) or not (ND) and trampling (T) or not (NT). Data are mean \pm SE.

Morphology and growth responses

Trampling increased the length and basal diameter of the main stem in connected ramets, but decreased them in disconnected ones, as reflected by the significant trampling \times severing effect on these traits (Table 1; Fig. 2a,c). Moreover, trampling significantly increased the deflection angle of the stems, which was reflected in a smaller height above the sand, i.e., vertical height. This effect was stronger in the disconnected than in the connected ramets (Fig. 2b, Table 1: significant trampling \times severing effect). Both severing and

defoliation significantly decreased basal diameter of the stems (Table 1; Fig. 2c).

Trampling significantly decreased stem mass in disconnected ramets, but had no significant effect in connected ramets (Fig. 3a, Table 1: significant trampling \times severing effect). Trampling increased fibrous root mass in connected ramets, but significantly decreased it in disconnected ramets (Fig. 3c, Table 1: significant trampling \times severing effect). Trampling significantly decreased leaf mass in both connected and disconnected ramets (Table 1, Fig. 3b). Defoliation reduced leaf mass, stem mass and leaf area ratio (Table 1; Fig. 3a,b,d), but did not interact with severing or trampling (Table 1). Severing significantly decreased leaf mass and stem mass of the surviving ramets (Table 1; Fig. 3a,b).

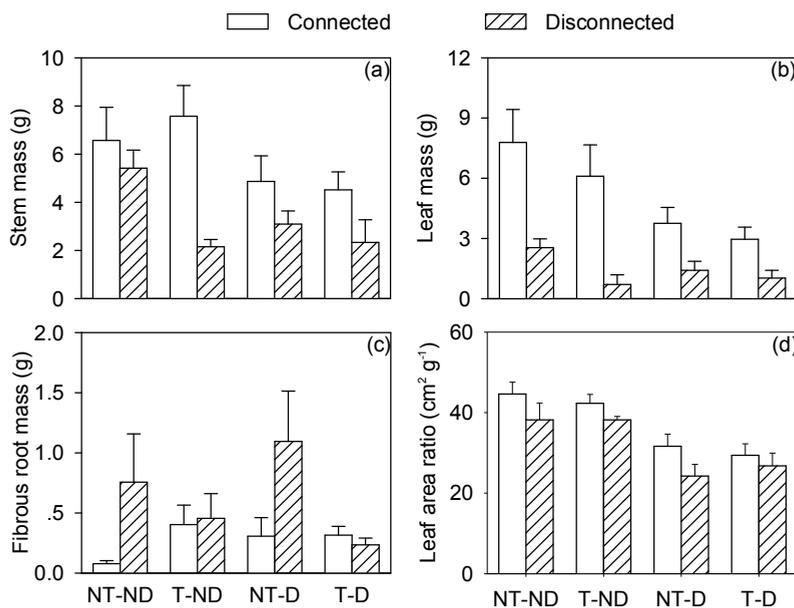


Fig. 3. Dry mass of stems (a), leaves (b), fibrous roots (c) and leaf area ratio (d) of the large *Populus simoni* ramets subjected to root severing (disconnected) or not (connected), defoliation (D) or not (ND) and trampling (T) or not (NT). Data are mean \pm SE.

Mechanical properties

In connected ramets, trampling significantly increased Young's modulus (E), second moment of area (I), flexural stiffness (EI), breaking stress (σ_b) and the minimum force needed to break (F_{break}) and bend (F_{bend}) stems, but in

disconnected ramets it decreased them (Fig. 4a-e, Table 1: significant trampling \times severing effect). Trampling markedly decreased $F_{\text{break}} / F_{\text{bend}}$ in both connected and disconnected ramets (Table 1; Fig. 4f). Defoliation significantly reduced I , EI , F_{break} and F_{bend} (Table 1; Fig. 4a,c,e), which are all a function of stem diameter (Eqns. 1, 3), but it did not interact with severing or trampling (Table 1).

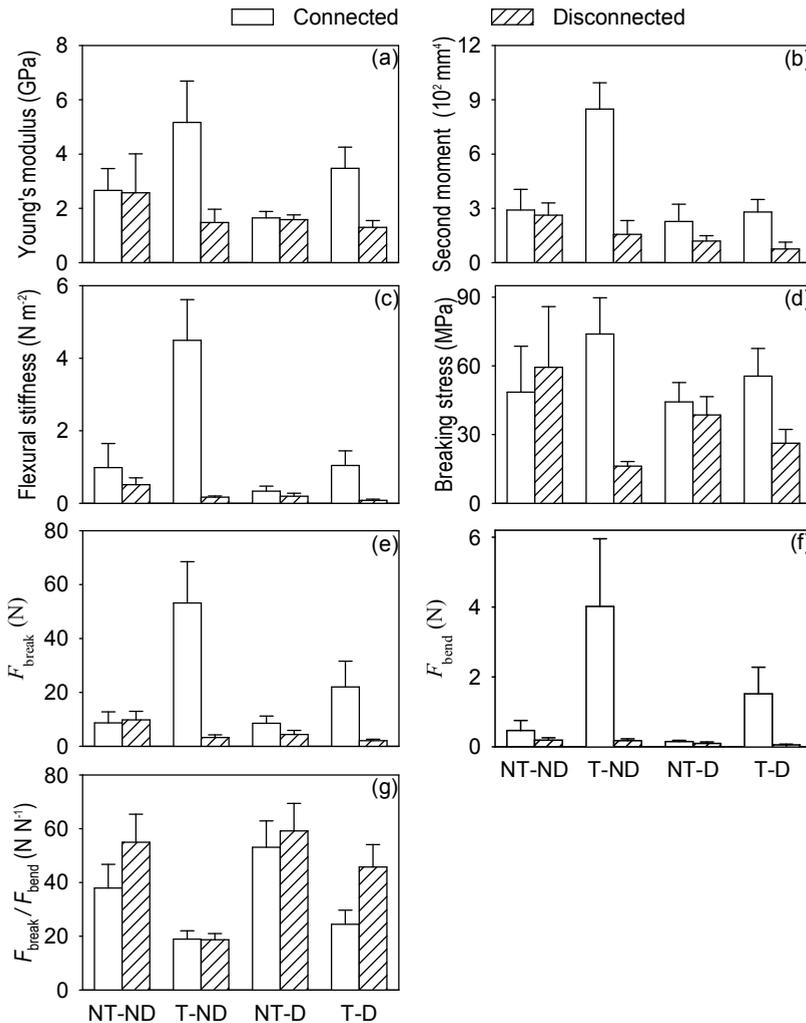


Fig. 4. Young's modulus (a), second moment of area (b), flexural stiffness (c), breaking stress (d), minimum lateral breaking force (e), bending force (f) and $F_{\text{break}} / F_{\text{bend}}$ (g) of the large *Populus simonii* ramets subjected to root severing (disconnected) or not (connected), defoliation (D) or not (ND) and trampling (T) or not (NT). Data are mean \pm SE.

Discussion

Effects of trampling and severing on survival

Successful colonization in dry sandland has often been associated with a clonal growth form, the argument being that the exchange of resources through clonal connections facilitates the establishment of vegetative offspring (Guàrdia *et al.*, 2000; Yu *et al.*, 2004; Yu *et al.*, 2008; Li, 2010). Demographic studies have further documented that population growth of clonal species in drylands is mainly governed by successful establishment of vegetative offspring (Li, 2010). However, experimental evidence for the role of clonal integration in determining growth and survival of vegetative offspring in drylands is limited (Yu *et al.*, 2004; Yu *et al.*, 2008). Both severing and trampling strongly decreased survival rates of *P. simonii*, particularly in small plants, but defoliation did not have a significant effect. These results suggest that clonal integration and the associated exchanges of resources between ramets strongly determined survival of new *P. simonii* ramets. They also indicate that the negative effects of grazing on survival of vegetative offspring are mediated by trampling rather than defoliation associated with grazing.

Growth and morphological responses to trampling, defoliation and severing

For connected ramets of *P. simonii*, repeated trampling not only increased stem length and basal diameter, but also increased deflection of stems from vertical (plants became greater vertically inclined). As a result, vertical heights above the soil surface were significantly reduced. These findings are consistent with studies on other woody plants (Sun & Liddle, 1993a; Andrés-Abellán *et al.*, 2006). For disconnected ramets, however, trampling decreased stem length, basal diameter, vertical height and stem mass. These results indicate that clonal integration can strongly modify plant responses to trampling. Clonal integration has been associated with tolerance to many stress factors, but its role in modifying responses to mechanical stress in general and trampling in particular has received little attention.

The interaction between clonal integration and trampling could have important functional significance in the study system. First, the study area has been under heavy grazing, and *P. simonii* has been planted to restore overgrazed lands. Second, the probability of being trampled as well as its associated impact strongly declines with increasing size (as also shown in our study); beyond a certain height plants are unlikely to be trampled. Thus, larger ramets that suffer little or no trampling can mitigate the damage imposed by trampling on their small vegetative offspring.

Defoliation negatively affected growth, and the final biomass was reduced by about 50%, which is proportional to the fraction of leaves that was removed (also 50%). This indicates that compensatory growth in *P. simonii* was very limited (see Anten *et al.*, 2003). Also, we did not find any interactive effect of trampling and defoliation. These results suggest that effects of trampling and defoliation are additive. The magnitude of the defoliation effect was similar among connected and disconnected plants, suggesting that clonal integration does not modify effects of defoliation on *P. simonii*. Thus, the role of clonal integration in increasing grazing tolerance may act through its effects in mitigating trampling effects rather than defoliation effects, which was never reported before.

We suggest that the effects of clonal integration, trampling and defoliation are at least partly mediated by water supply. In the study area, precipitation is low (260–450 mm annually) and ramets of *P. simonii* grew in almost pure sand. Thus, the young ramets most likely experienced water limitations. In theory, a given ramet can obtain water from its own fibrous roots, from its mother ramet or other ramets through the main connecting root. Due to the shallow root system and sand movement, these roots can easily become exposed, making them vulnerable to drying or damage. Interestingly, 16% of the ramets that died during the experiment had not yet produced fibrous roots, while all the surviving ramets had. For the surviving ramets, those that were disconnected tended to have more fibrous roots (0.70 ± 0.19 g) than those that were connected (0.27 ± 0.06 g), suggesting that the amount of fibrous roots is important in determining survival of disconnected ramets. Disconnected, trampled plants had considerably smaller rooting systems than non-trampled ones (0.33 ± 0.09 vs. 0.96 ± 0.29 g), suggesting that trampling caused either reduced root growth or damage to existing roots. This effect could be more severe for *P. simonii* because it is woody and thus has relatively rigid stems. As a result, trampling induced a large bending moment on the root system. Fibrous roots in sandy soils tend to have low shear strengths (Berry *et al.*, 2004). Together these factors contributed to the low root anchorage strength (Crook & Ennos, 1994; van Delden *et al.*, 2010). Thus we suggest that trampling exacerbated drought stress by causing root damage, and that this effect could be mitigated by water transported through clonal connections.

Effects of trampling and severing on mechanical traits

Plants can prevent mechanical damage either by increasing their resistance to breakage or by enhancing their flexibility to minimize the forces encountered (Puijalón *et al.*, 2008; Puijalón *et al.*, 2011; Paul-Victor & Rowe, 2011). At the

stem level, the former can be represented by the minimum breaking force (F_{break}) and the latter is the inverse of the force needed to bend the stem ($1/F_{\text{bend}}$). Across species there seems a tight negative correlation between F_{break} and F_{bend} , indicating that plants cannot maximize both flexibility and strength (Puijalon *et al.*, 2011). Because trampling entails an overwhelming force, flexibility rather than strength should be expected to increase trampling tolerance (Sun & Liddle, 1993a). This is true for disconnected ramets of *P. simonii*: trampling reduced stem diameter and breaking stress, which resulted in a reduction in both stem strength (F_{break}) and resistance to bending (F_{bend}).

For connected ramets, however, we observed clear opposite responses. Trampling increased stem diameter and associated flexural stiffness and as a result increased both F_{break} and F_{bend} . These results support the previously noted point that clonal integration can strongly modify plant responses to trampling. Moreover, trampling increased bending resistance more than strength as reflected in the reduced value of $F_{\text{break}} / F_{\text{bend}}$. These results are consistent with the observed responses of plants to other forms of mechanical stress (e.g., wind, rubbing and flexing; Biro *et al.*, 1980; Telewski, 1990; Anten *et al.*, 2005). It is possible that in *P. simonii* thigmomorphogenic responses induced by trampling are similar to those induced by other forms of mechanical stress such as wind, and such responses may reduce the chance of mechanical damage and thus enhance trampling resistance (Anten *et al.*, 2005).

Conclusions

Although a high relative frequency of rhizomatous species is known to be associated with intense grazing pressure (van Staalduinen *et al.*, 2007), our study is the first to document that clonal integration may contribute to grazing tolerance by mitigating the effects of trampling. This interactive effect may be mediated by the capacity for the transport of water or other key resources through clonal connections, which partly compensates for the trampling-induced damage to the root system. This observation adds to our understanding of plant adaptation to grazing by suggesting that anti-herbivore tolerance in clonal plants is at least partly related to the ability to withstand trampling rather than defoliation.

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Populus simonii in the mobile dune.

Chapter 4

Effects of trampling on morphological and mechanical traits of drylands shrubs species do not depend on water availability

with Sofia M. A .Freitas, Fei-Hai Yu, Ming Dong, Niels P. R. Anten and Marinus J. A. Werger

Abstract

- In semiarid regions trampling by large herbivores and water availability are two factors limiting plant growth and distribution. Trampling can strongly affect plant performance, but little is known about responses of morphological and mechanical traits of woody plants to trampling and their possible interaction with water availability.
- Seedlings of four shrubs (*Caragana intermedia*, *Cynanchum komarovi*, *Hedysarum laeve* and *Hippophae rhamnoides*), common in the semiarid Mu Us Sandland, were grown at 4% and 10% soil water content and exposed to either simulated trampling or not. Growth, morphological and mechanical traits were measured.
- Trampling decreased vertical height and increased basal diameter and stem resistance to bending and rupture (as indicated by the increased minimum bending and breaking force) in all four species. Increasing water availability increased biomass, stem length, basal diameter, leaf thickness and rigidity of stems in all four species except *C. komarovii*. However, there were no interactive effects of trampling and water content on any of these traits among species except for minimum bending force and the ratio between stem resistance to rupture and bending.
- Overall shrub species have a high degree of trampling resistance by morphological and mechanical modifications, and the effects of trampling do not depend on water availability. However, the increasing water availability can also affect trade-off between stem strength and flexibility caused by trampling, which differs among species. Water plays an important role not only in growth but also in trampling adaptation in drylands.

Key words: Drylands; growth; morphology; shrub; trampling; water content.

Introduction

Trampling by large grazers is common in arid and semiarid ecosystems, and may strongly impact plant performance (Rusterholz *et al.*, 2009; Sørensen *et al.*, 2009; Xu *et al.*, 2012), community structure (Kobayashi *et al.*, 1997; Malmivaara-Lämsä *et al.*, 2008; Hamberg *et al.*, 2010) and ecosystem functioning (Dunne *et al.*, 2011). Trampling can directly cause tissue loss and damage and indirectly impose mechanical stress on plants (Olf & Ritchie, 1998; Xu *et al.*, 2012). In arid and semiarid regions trampling also contributes to desertification (Ibáñez *et al.*, 2007).

Phenotypic responses of plant growth and morphology to trampling have received increasing attention (Kobayashi *et al.*, 1999; Littlemore & Barker, 2003; Rodgers *et al.*, 2003; Sørensen *et al.*, 2009; Xu *et al.*, 2012). Trampling commonly decreases plant height (Sun & Liddle, 1993a; Andrés-Abellán *et al.*, 2006; Hill & Pickering, 2009; Hill & Pickering, 2009), leaf size (Sun & Liddle, 1993a) and seed size (Meerts & Garnier, 1996). Also, different species respond differently to trampling (Kobayashi *et al.*, 1999; Littlemore & Barker, 2003; Rodgers *et al.*, 2003), and the differences tend to be associated with growth forms (Sun & Liddle, 1993a; Sun & Liddle, 1993b; Kobayashi *et al.*, 1999). For instance, erect growing plant species tend to be less tolerant to trampling than species with a rosette, tussock or otherwise prostrate growth form (Sun & Liddle, 1993b; Kobayashi *et al.*, 1999; Littlemore & Barker, 2003).

However, phenotypic responses of mechanical properties such as stem flexibility, leaf toughness and root strength to trampling have received much less attention, although such responses are likely to strongly contribute to trampling tolerance (Kobayashi *et al.*, 1999; Striker *et al.*, 2011; Xu *et al.*, 2012). Trampling can impose strong mechanical stress on plants (Olf & Ritchie, 1998; Xu *et al.*, 2012) that results in increased toughness of stems and leaves (Sun & Liddle, 1993b; Kobayashi *et al.*, 1999). Contrary to other forms of mechanical stress such as wind, touch or rubbing that are relatively gentle and well studied, trampling often entails overwhelmingly large forces that plants hardly resist. Stem stress avoidance through increased flexibility is then the only viable response for small or young plants. However, the little work that has been done in this respect has focused mostly on grasses (Sun & Liddle, 1993a; Sun & Liddle, 1993b; Kobayashi *et al.*, 1999; Littlemore & Barker, 2003; Rodgers *et al.*, 2003; Hill & Pickering, 2009), and in this respect we know almost nothing about woody species (Xu *et al.*, 2012).

In arid and semiarid ecosystems, water is another important factor limiting

plant performance (Hanke *et al.*, 2011). It is thus important to determine the extent to which trampling effects on plants are affected by water availability. Increasing water availability can increase allocation to stems, which may increase rigidity, height and diameter and thus resistance to trampling. High water availability also decreases allocation to roots, which may greatly increase the effects of trampling on the fixation of the root system known as anchorage strength of plants (Goodman & Ennos, 1996). However, to our knowledge, no study has addressed how water availability can modify effects of trampling on growth, morphology and mechanical traits of plants. We hypothesize that increasing water availability can change toughness and flexibility of plants' stem, which affects resistance of plants to trampling.

The Mu Us Sandland in north China is a typical semiarid area where overgrazing by large domestic animals is common and desertification is severe (Zhang, 1994). A large number of shrub species are distributed in the Mu Us Sandland, including some relic plants (Zhang, 1994); these shrubs play important roles in protection of local ecosystems. Because of the abundance in shrub species, the Mu Us Sandland is called “kingdom of shrubs” (Dong *et al.*, 1997). We conducted a greenhouse experiment with four shrub species (*Caragana intermedia*, *Cynanchum komarovi*, *Hedysarum laeve* and *Hippophae rhamnoides*) typical of the Mu Us Sandland. Plants were exposed to two levels of soil water content (4% and 10% soil water content) and two levels of trampling (with vs. without). Specifically, we addressed the following questions: (1) What are the effects of trampling on the growth, morphological and mechanical traits of the four species, and do trampling effects depend on the species identity? (2) Can water availability modify the effects of trampling on plants?

Materials and methods

Study species

The four species used in this experiment are common and widely distributed in the Mu Us Sandland (37°30'-39°20'N, 107°20'-111°30'E), a semiarid area where is main part of Ordos plateau in northern China (Zhang, 1994). Because of human activity and dry condition of the sandland, degradation of local vegetation and desertification are quite severe (Zhang, 1994). *Caragana intermedia* Kuanget (Leguminosae) and *Hippophae rhamnoides* L. (Elaeagnaceae) are shrubs and *Cynanchum komarovii* Al. (Asclepiadaceae) and *Hedysarum laeve* Maxim. (Leguminosae) are semi-shrubs (Jiang & Li, 1977; Fang, 1983; Fu, 1993; Cui, 1998). *H. rhamnoides* and *H. laeve* are

capable of clonal growth by rhizomes (He *et al.*, 2006; Zhang *et al.*, 2001). Except of *C. komarovii*, the other three species are widely used in vegetation recovery and control of sand dunes expansion in this area, palatable to animals and other abilities. *C. komarovii* is poisonous to animals, and common in degraded lands, and is an important indicator of desertification (Jiang & Li, 1977; Fang, 1983; Fu, 1993; Cui, 1998).

The experiment

Seeds of four species were collected from July to October of 2009 near the Ordos Sandland Ecological Research Station (OSES, 39°29'37.6"N, 110°11'29.4"E, 1300 m a.s.l.) of the Institute of Botany, the Chinese Academy of Sciences, located in the Mu Us Sandland in Inner Mongolia, China. On 21 December 2009, approximately 400 seeds per species were placed on the surface of shallow plastic square boxes (30 cm × 15 cm × 5 cm, 2.25 L) filled with sand and underwent a stratification period of two weeks at a temperature of 5 °C. In January 2010, germinated plants were transplanted to small square pots (7 cm × 7 cm × 8 cm, 0.24 L). After another three months of growth, seedlings of similar sizes were transplanted to 6.5 L pots (24 cm in diameter and 21 cm in height) fully filled with sand. Two extra grams of solid fertilizer (16N-11P₂O₅-11K₂O-3MgO + trace elements, 3-4 months, Osmocote Exact, Scotts International B.V, Heerlen, Netherlands) were added to each pot and mixed evenly with sand. On 12 May 2010, 48 of *C. intermediate* and 52 of each other three species in similar size were chosen for the experiment.

On 13 May 2010, the seedlings of each species were randomly subjected to one of four combined treatments including two levels of soil water availability (4% vs. 10% water content for dry and wet conditions, see Appendix) and two levels of trampling (with vs. no). During the experiment the volumetric soil water content was maintained nearly constant at 4% or 10% by periodical measurement with a Time Domain Reflectometer (Wageningen, the Netherlands). Trampling was applied with a manually constructed load whose weight (6.22 kg) and surface area (3.07 cm in diameter) were such that, when placed on a plant, it produced a stress level (0.84 kg cm⁻²) similar to that produced by the hoof of a 40 kg sheep (Lin *et al.*, 2008). The load consisted of one long metal pipe and four short metal pipes. The short pipes were fixed tightly with tape and rope to the middle of the long pipe. Pipes were filled with concrete to obtain the appropriate weight. After that pipes were sealed well with tape to avoid leakage. A cap with the diameter of 3.07 cm was installed at the bottom of the long pipe. The trampling was applied three times a week for 10 seconds each time. The trampled direction was changed each

time. Positions of pots were altered every two weeks to minimize the effect of the spatial heterogeneity in the greenhouse.

The experiment was carried out in a climate controlled greenhouse compartment located at the Utrecht University Botanical Gardens, the Netherlands (52° 5' 16.79"N, 5° 10' 8.26"E). The average temperature was 18°C and the average humidity 40%.

Measurements

On 20 June 2010, all experimental plants were harvested. Stem length, vertical height from soil surface to the top of trampled plants, basal diameter and leaf thickness of main shoots were measured. Main shoots of plants were separated into leaves, stems and roots. Leaf area was measured with a LI-3100 leaf area meter (LI-COR, Inc. Lincoln, Nebraska, USA). Aboveground shoots were packed in wet tissue to avoid turgor loss and taken to the lab to measure mechanical traits. Dry mass of different organs were determined after drying at 70°C for 48 h. We only measured the whole dry mass of the new shoots, including leaves and stems.

We determined four mechanical traits: Young's modulus (E), breaking stress (σ_b), yield stress (σ_y) and maximum load force of main stems using a universal electromechanical testing machine (Type 5542, Instron, Norwood, Massachusetts, USA) and applying the three-point bending technique (for details see Anten *et al.*, 2009). Vertically applied forces (F ; N) and resulting deflections (δ ; m) were recorded. The distance between supports was adjusted such that it was always approximately 15 times the diameter of the stem section.

We calculated leaf mass area (LMA; g cm^{-2}) and leaf mass density (LMD; g cm^{-3}) to describing the leaf characteristics. To present the mechanical traits of plants' stems, a number of parameters were measured and calculated, including: the second moment of area (I ; m^4) describing the geometric contribution to stiffness of the stem (Jaffe *et al.*, 1984; Niklas, 1996); the Young's modulus (E) representing stiffness of an elastic material (Gere & Timoshenko, 1999; Anten *et al.*, 2005); flexural stiffness (EI ; N m^2), which is the rigidity of a stem cross section; the stem yield stress (σ_y) the stress level at which a structure shifts from elastic to plastic (permanent) bending; and the breaking stress (σ_b), which quantifies the resistance of stem tissue to rupture. Details of calculations of these mechanical traits can be found in Anten *et al.* (2005).

The stem traits described above were used to calculate a number of parameters that indicate whole-stem behavior under externally applied forces.

We note that these are qualitative measures of stem mechanical behavior solely used for comparative purposes. We calculated the minimum bending force (F_{bend}) referring to the force required to bend the stem by a small specified angle, and the minimum lateral breaking force (F_{break}) required to rupture the stem at its base. In this study we assume that trampling exerts an overwhelmingly large force on plants and consider a favorable balance between stem strength to bending bending resistance to reduce the chance of damage. We use $F_{\text{break}} / F_{\text{bend}}$ as a simple proxy to describe this balance, which shows that the balance between strength and flexibility is related to breaking stress, shoot vertical height, Young's modulus and basal diameter (details in Xu *et al.*, 2012).

Table 1. Results of ANOVA for the effects of species (S), trampling (T), water (W) and their interactions on morphological and growth properties of four species

	Species	Trampling	Water	S×T	S×W	T×W	S×T×W
Stem length	69.82 ^{***}	13.91 ^{***}	181.96 ^{***}	3.22 [*]	20.74 ^{***}	3.85 ^{ns}	1.82 ^{ns}
Vertical height	14.01 ^{***}	164.12 ^{***}	95.44 ^{***}	9.59 ^{***}	13.84 ^{***}	1.15 ^{ns}	2.94 [*]
Diameter	194.68 ^{***}	37.77 ^{***}	64.17 ^{***}	0.59 ^{ns}	10.63 ^{***}	0.53 ^{ns}	0.87 ^{ns}
Leaf thickness	194.86 ^{***}	0.15 ^{ns}	14.16 ^{***}	0.10 ^{ns}	2.30 ^{ns}	0.18 ^{ns}	0.89 ^{ns}
LMA	1.89 ^{ns}	6.24 [*]	6.17 [*]	0.44 ^{ns}	0.36 ^{ns}	1.15 ^{ns}	1.03 ^{ns}
LMD	45.40 ^{***}	2.42 ^{ns}	15.67 ^{***}	0.22 ^{ns}	0.96 ^{ns}	0.03 ^{ns}	0.89 ^{ns}
Biomass	61.58 ^{***}	2.72 ^{ns}	94.82 ^{***}	2.70 [*]	18.41 ^{***}	2.41 ^{ns}	2.66 ^{ns}
Stem ratio	31.08 ^{***}	0.51 ^{ns}	19.29 ^{***}	3.76 [*]	6.26 ^{***}	1.88 ^{ns}	0.60 ^{ns}
Root ratio	84.43 ^{***}	0.64 ^{ns}	440.31 ^{***}	0.88 ^{ns}	28.76 ^{***}	0.03 ^{ns}	0.57 ^{ns}
Leaf ratio	84.73 ^{***}	1.61 ^{ns}	324.32 ^{***}	1.89 ^{ns}	21.58 ^{***}	3.50 ^{ns}	0.43 ^{ns}
New shoot ratio	51.35 ^{***}	0.02 ^{ns}	80.88 ^{***}	1.86 ^{ns}	25.25 ^{***}	0.32 ^{ns}	0.38 ^{ns}

F values and significance levels (^{***} $P < 0.001$, ^{**} $P < 0.01$, ^{*} $P < 0.05$ and ^{ns} $P \geq 0.05$) are given; degree of freedom from all the effects are (1, 175). Data were Ln-transformed before analyses.

Statistical analysis

A three-way ANOVA was used to test the effects of species, trampling and water availability on growth, morphological and mechanical traits. Before

analysis, data were checked for equality of variance with Levene's test and for normality Shapiro-Wilk's test. SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA) was employed for the analyses. Here we chose $P < 0.05$ as significance level.

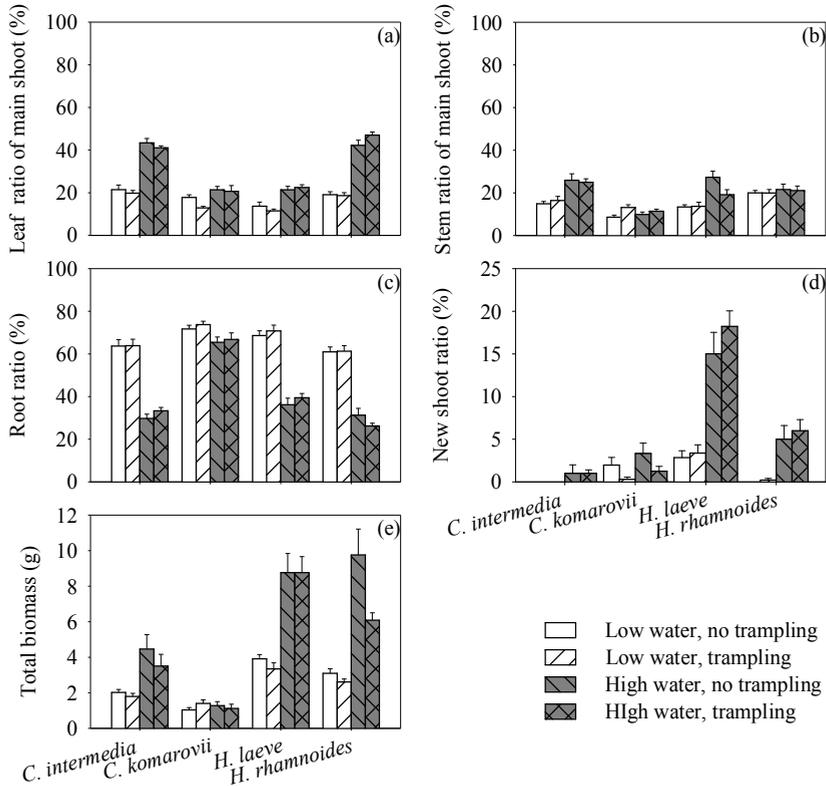


Fig. 1. Leaf mass of main shoot ratio (a), stem mass of main shoot ratio (b), root ratio (c), new shoot ratio (d) and total biomass (e) of four species. Data are mean \pm SE without transformation.

Results

Growth and morphological traits

Trampling decreased biomass of *H. rhamnoides* but had no significant effect in the other three species (Table 1; Fig. 1e). The fractional biomass allocation to different plant parts was not significantly affected by trampling, except in *C. komarovii* where stem allocation increased (Table 1; Fig. 1b). Increasing water content increased final biomass in all species except *C. komarovii* (Table 1; Fig. 1e). At low water content, more biomass was allocated to roots in all four species, at the expense of allocation to other organs (Table 1; Fig. 2).

Trampling significantly reduced the main stem length in all species except of *C. komarovii* (Table 1; Fig. 2a) and caused stems to be less inclined, thus reducing the distance between soil level and stem apex (i.e., vertical height), albeit less strongly in *C. intermedia* and *C. komarovii* than in the other two species (Table 1; Fig. 2b). Trampling consistently increased stem diameter (Table 1; Fig. 2c). Plants in high water availability had larger stem length, vertical height and diameter in all species except *C. komarovii* (Table 1; Fig. 1a, b, c).

Trampling did not significantly affect leaf thickness (Table 1; Fig. 2d) or leaf mass density (LMD; Fig. 2f), but significantly decreased leaf mass per unit area (LMA; Fig. 2e). Increasing water availability increased leaf thickness (Table 1; Fig. 2d) and decreased LMA (Fig. 2e) and LMD (Fig. 2f) of all four species except *C. komarovii*.

Table 2. Results of ANOVA for the effects of species (S), trampling (T), water (W) and their interactions on mechanical properties of four species.

	Species	Trampling	Water	S×T	S×W	T×W	S×T×W
Young's modulus	40.00 ^{***}	22.04 ^{***}	4.21 [*]	0.31 ^{ns}	2.11 ^{ns}	0.05 ^{ns}	0.91 ^{ns}
Second moment of area	167.46 ^{***}	19.33 ^{***}	63.84 ^{***}	0.66 ^{ns}	11.44 ^{***}	0.01 ^{ns}	1.16 ^{ns}
Flexural stiffness	117.89 ^{***}	9.02 ^{**}	54.75 ^{***}	0.07 ^{ns}	8.14 ^{***}	0.17 ^{ns}	0.40 ^{ns}
Yield stress	11.40 ^{***}	0.28 ^{ns}	16.57 ^{***}	1.14 ^{ns}	4.61 ^{**}	1.98 ^{ns}	0.16 ^{ns}
Breaking stress	36.18 ^{**}	5.05 [*]	5.70 [*]	0.07 ^{ns}	4.84 ^{**}	0.04 ^{ns}	0.41 ^{ns}
F_{break}	54.87 ^{***}	29.26 ^{***}	4.43 [*]	0.15 ^{ns}	4.75 ^{**}	0.03 ^{ns}	1.03 ^{ns}
F_{bend}	56.25 ^{***}	31.31 ^{***}	0.61 ^{ns}	0.57 ^{ns}	2.76 [*]	0.55 ^{ns}	3.98 [*]
F_{breakl}/F_{bend}	13.29 ^{***}	6.82 ^{**}	4.98 [*]	2.13 ^{ns}	2.47 ^{ns}	0.94 ^{ns}	7.39 ^{***}

F values and the significance levels (^{***} $P < 0.001$, ^{**} $P < 0.01$, ^{*} $P < 0.05$ and ^{ns} $P \geq 0.05$) are given; degree of freedom from all the effects are (1, 175). Data were Ln-transformed before analyses.

Mechanical properties

Trampling reduced tissue rigidity expressed as the Young's modulus (E) for all four species, in both dry and wet conditions (Table 2; Fig. 3a). However, trampling increased basal diameter (Table 1; Fig. 2c) which strongly determines the second moment of area (I ; Table 2; Fig. 3b). The product of E and I , flexural stiffness (EI) exhibited a similar trend as I (Table 2; Fig. 3c), indicating that variation in I contributed more to flexural stiffness than

variation in E . Compared with the other three species, stems of *C. komarovii* had very low flexural stiffness, which could be attributed to the thinner stems with a lower I . Trampling also resulted in a consistent but small reduction in the breaking stress (σ_b), but had no significant effect on the yield stress (σ_y ; Table 2; Fig. 3d, e). In *H. laeve* high water availability had a strong positive effect on σ_b and σ_y values. The effect of soil water content on tissue properties clearly differed between species (Table 2). Low water content reduced E in *C. intermedia* and *H. laeve* but not in the other two species. Similarly it reduced σ_b and σ_y only in *H. laeve*.

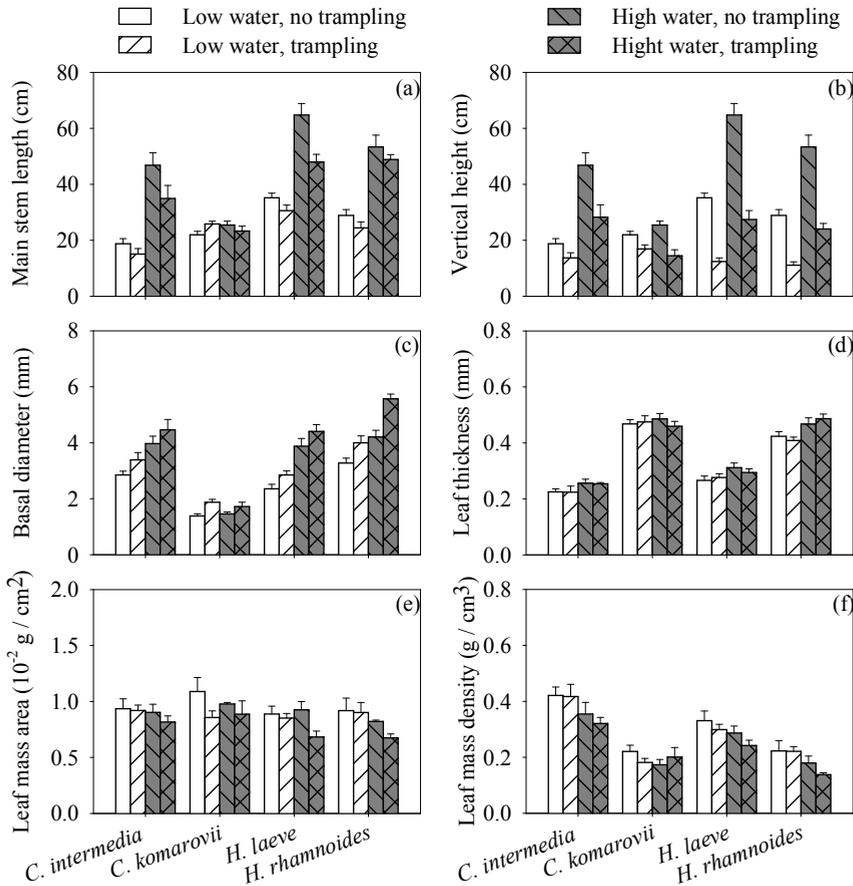


Fig. 2. Main stem length (a), vertical height (b), basal diameter (c), leaf thickness (d), leaf mass area (e) and leaf mass density (f) of four species. Data are mean \pm SE without transformation.

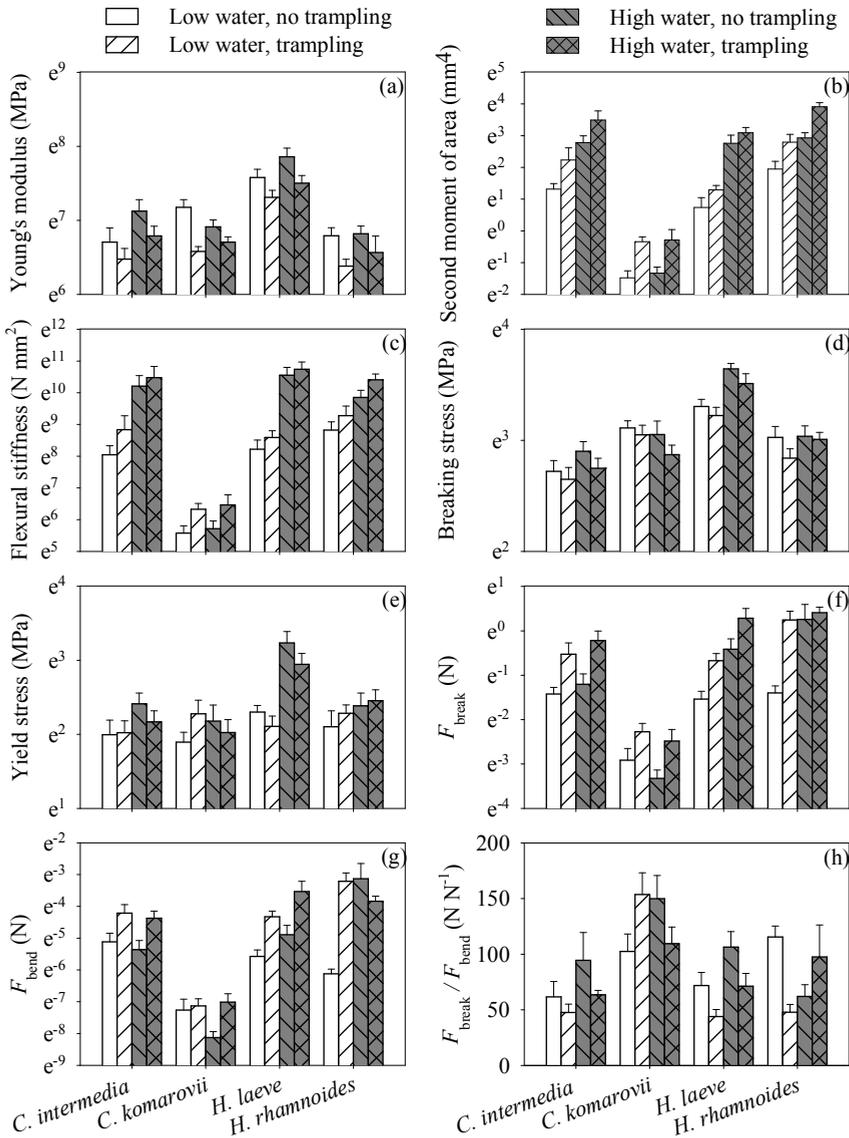


Fig. 3. Young's modulus (a), second moment of area (b), flexural stiffness (c), yield stress (d), breaking stress (e), minimum lateral breaking force (F_{break}) (f), bending force (F_{bend}) (g) and $F_{\text{break}}/F_{\text{bend}}$ (h) of four species. Data are mean \pm SE. Except for h, values of y axis are Ln-transformed as a result of low values of *C. komarovii*.

To determine stem resistance to either breakage or bending we calculated the minimum forces required to either rupture the stem at its base (F_{break}) or start to bend it (F_{bend}). F_{break} thus indicates stem strength and F_{bend} resistance to bending. Trampling increased both F_{break} and F_{bend} across all species and both water treatments (Table 2; Fig 3f, g). The ratio $F_{\text{break}} / F_{\text{bend}}$, proxy for the balance between strength and flexibility, of *C. intermedia* and *H. laeve* was decreased by trampling (Table 2; Fig. 3h). Trampling enhanced the ratio in *C. komarovii* in low water content but reduced it in high water content and with the opposite *H. rhamnoides* (Table 2; Fig. 3h).

Discussion

Most studies on trampling are at the community level (Liddle, 1975; Bowles & Maun, 1982; Kutiel *et al.*, 2000), and few have been conducted at the individual plant level. However, in mobile or semi-fixed dunes in drylands the vegetation is sparse with plants tending to grow individually, such that studies on the effects of trampling on individual plants is more practical. The trampling treatment used in our study simulated a relatively frequent impact of sheep, the most common grazer in the Mu Us Sandland. We found that trampling had no effect on the survival of the seedlings of all the four species, and had little effect on final biomass, suggesting that juveniles of these woody species are tolerant to trampling.

Trampling decreased stem length and vertical height of the four shrub species, agreeing with previous findings on herbaceous species (Sun & Liddle, 1993a; Andrés-Abellán *et al.*, 2006; Hill & Pickering, 2009; Hill & Pickering, 2009). suggesting that reduced stem elongation is a common responses to trampling. Under trampling, plants may generate various other changes in morphological and mechanical traits on the whole-plant level, including decreases in leaf size (Sun & Liddle, 1993a) and seed size (Meerts & Garnier, 1996) and increases in stem and leaf toughness (Sun & Liddle, 1993b; Kobayashi *et al.*, 1999). However, our results of leaf characteristics are different from others, very likely because trampling was directly imposed on stem in our study, but on the whole plant as in other studies (Sun & Liddle, 1993a). Reduction in stem elongation (except of *C. komarovii*) and declination by trampling together resulted in a smaller height of the stem apex from the soil level (H_v). Trampling also increased radial stem growth. Thicker stem can resist larger forces. Therefore variances in diameter may contribute to a larger flexural rigidity and a larger resistance to bending and rupture.

Contrary to our expectation, the effects of trampling on the mechanical

traits of the four shrub species did not depend on water availability. In a previous study, the effects of shaking (another type of mechanical stress) on the stem rigidity of *H. laeve* was also independent of water availability (Wang *et al.*, 2008). However, for the annual herb *Corispermum mongolicum*, it was found that shaking reduced stem tissue rigidity at low water availability but increased it at high water availability (Wang *et al.*, 2009). Therefore, it seems that the extent to which plant responses to mechanical stress are mediated by water availability depends on the growth form of plants (i.e., them being woody or herbaceous). Turgor pressure plays a more important role in determining stem rigidity in herbaceous plants than in woody plants (Struik, 1965). In woody plants stem mechanical traits are less sensitive to the water status of the stem. Mechanical responses of woody species to mechanical stress are likely to depend more on the composition and quantity of tissue (e.g. parenchyma, sclerenchyma, xylem, vascular tissue) than on turgor pressure. However, plant resistance to external forces depends on both mechanical and morphological traits at the whole stem level. Though increasing water availability did not affect stem tissue rigidity, it strongly increased stem basal diameter which in turn resulted in a larger whole-stem resistance to both bending and rupture .

Plants can prevent mechanical damage by building a flexible stem that easily reconfigures to minimize the amount of force encountered (stress avoidance), by building strong structures that resist large forces (stress tolerance) (Puijalón *et al.*, 2008; Puijalón *et al.*, 2011). A trade-off between strength and flexibility can be expected because thicker structures are stronger but less flexible than thinner structures (Puijalón *et al.*, 2011). Such a trade-off inevitably imposes constraints on the expression of traits associated with both strategies. Because trampling of small plants by large mammals represents an overwhelmingly large force, stress avoidance is most likely the most viable strategy to prevent damage. However, both trampling and water availability resulted in thicker stems, which is associated with larger stress tolerance through increased rupture and bending resistance . In that case, plants need to build a favorable balance between avoidance and tolerance. In the present study stress avoidance was characterized by the inverse of minimum bending force ($1/F_{\text{bend}}$), stress tolerance by the minimum breaking force (F_{break}) and the balance by the ratio of breaking and bending force ($F_{\text{break}}/F_{\text{bend}}$). Overall, we found that trampling enhanced both F_{break} and F_{bend} , meaning more force was needed to bend or break trampled shoots. The trampling induced increase in stem diameter and associated increases in F_{break} and F_{bend} are consistent with other researches about responses of plants to other forms of MS (e.g. wind,

rubbing and flexing; Biro *et al.*, 1980; Telewski, 1990; Anten *et al.*, 2005). These latter forms of MS usually entail smaller forces than trampling. In semi-arid regions wind is the predominant form of mechanical stress and it is possible that thigmomorphogenic responses in our species act to reduce wind damage rather than trampling damage.

On the other hand, generally trampling increased F_{bend} more, thus reduced values of $F_{\text{break}}/F_{\text{bend}}$, reflecting a less favorable balance between strength and flexibility. The exceptions are *C. komarovii* in the low water availability and *H. rhamnoides* in the high water availability, where trampling increased $F_{\text{break}}/F_{\text{bend}}$. As argued in the previous paragraph, this enhanced strength at the expense of flexibility in response to trampling seems maladaptive as trampling represents an overwhelming force. On the other hand the observed reduction in stem inclination angle may help reduce the chances of mechanical damage under trampling as the bending required to press the plant shoot to the soil becomes smaller.

In drylands, resources are highly heterogeneously distributed (Housman *et al.*, 2007), which possibly leads to a large range of variance in phenotypic plasticity, especially combined with other external stresses. In this study we find that effect of trampling on woody plants is independent of water content. Comparing with other studies (Wang *et al.*, 2008; Wang *et al.*, 2009), it is likely that interaction are probably more common in herbaceous than in woody species. However, the results also show that not only in growth, but also in trampling resistance, water plays an important role in drylands. Furthermore, the effects of water on trade-off of stem strength and flexibility caused by trampling differ among species.

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Appendix

Soil water characteristic

Our intention was to implement drought treatments in such a way that plants of different species and trampling treatments would experience very similar soil water potentials. Water potential is the energy associated with the forces to hold the water in the soil. For each particular soil there is a unique relationship between water content and water potential. Water potential is the standard to define the dry and wet condition while water content is easily monitored to reflect the water potential. Therefore, a small experiment was conducted to measure the relationship between the water potential and water content of sand to determine the levels of water treatments. Five square pots (13 cm×13 cm× 13 cm, 1.55L) were filled with dry sand which would be used in the following experiment. Sand was dried at 105 °C in the stove for one days. One gypsum block was inserted into each pot at 10 cm connecting to the soil moisture measuring system (Eijkelkamp, Giesbeek, the Netherlands). Weights of pots filled with dry sand and the gypsum block were measured before the measurement. As the pots for experiment were not very large, we considered sand of the whole pots had same water potential. Every hour, 30 ml water was added to the pot. The machine recorded the soil water potential while the water content of the soil mixture was gravimetrically (θ_g) estimated using the following equation:

$$\theta_g = \frac{m_{water}}{m_{soil}} = \frac{m_{wet} - m_{dry}}{m_{dry}} \quad (1)$$

where m_{wet} is weight of moisture soil and m_{dry} is weight of the same soil sample which was dried for two days at 105°C until weight became constant.

The volumetric water content (θ_v) was estimated as:

$$\theta_v = \frac{\theta_g * \rho_{sand}}{\rho_{water}} \quad (2)$$

where density of sand (ρ_{sand}) was measured with the dried sand, and density of water was treated as 1g / cm³.

According to the water characteristic curve of volumetric water content and water potential (Appendix Fig. 1) and other study (Martine *et al.*, 2008), the

values of 4% and 10% volumetric water content were chosen and correspond respectively to soil water potentials of -13 Bars (low soil water condition) and -2 Bars (high soil water condition).

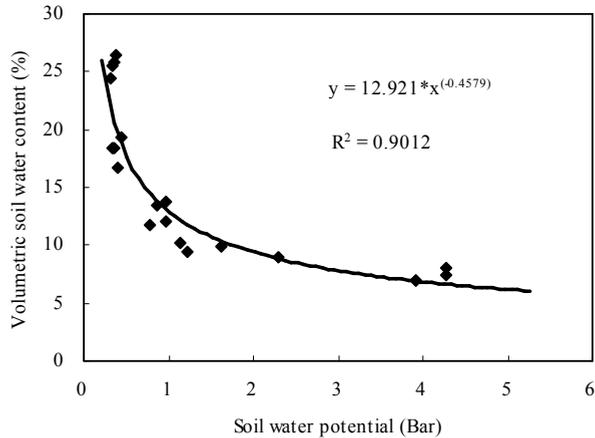


Fig. 1. Soil water characteristic describes the relationship between volumetric soil water content (%) and soil water potential (Bar). The dots are fitted and showed as $y = 12.921 * x^{(-0.4579)}$ ($R^2 = 0.9012$).



Seedling of *Caragana intermedia* in mobile dune



Cynanchum komarovi in mobile dune

Chapter 5

Intraspecific variation of a desert shrub species in phenotypic plasticity in response to sand burial

with Heidrun Huber, Heinjo J. During, Niels P. R. Anten

Abstract

· In drylands, sand burial is an important and common stress factor affecting growth and mechanical properties of plants. Shoot elongation is one of the main plastic responses of plants to burial. Yet, intra-specific variation in this response to burial and the extent to which this variation is functionally coordinated with variation in other trait responses (e.g. stem diameter and tissue stiffness) has not been well investigated.

· Seedlings of the desert shrub species *Caragana intermedia* belonging to 18 different seed families were either or not subjected to repeated sand burial up to 2/3 of the length of the stem. Height and basal diameter were measured five times during the experiment to determine their growth rate. At final harvest, leaf properties, mechanical traits of stems and dry biomass were determined. We also calculated plasticity in response to burial by comparing buried and unburied plants.

· Burial significantly increased the shoot elongation rate of plants and stem diameter growth rate, but decreased biomass increment and the flexural rigidity of the basal cross section. Plants from different seed families differed significantly with respect to shoot elongation rate, leaf morphology, and mass. The effects of burial on mechanical stem traits (tissue stiffness and strength, and stem flexural rigidity) differed significantly among seed families.

· The results of this study indicate that plasticity in different stem traits in response to sand burial is functionally coordinated with respect to mechanical stability. The results emphasize the importance of considering functionally coordinated traits when analyzing the consequences in phenotypic plasticity in plants.

Key words: Biomass; biomechanics; phenotypic plasticity; sand burial; seed family; shoot elongation.

Introduction

In many drylands areas, sand movement by strong winds is a common phenomenon (Zhang, 1994; Yu *et al.*, 2004; Yu *et al.*, 2008). This sand movement can either cover the vegetation (burial hereafter and the focus of this paper) or conversely uncover and expose the roots (denudation). Burial may have a number of stressful effects on plants: coverage of leaves and thus a reduction of exposed photosynthetically active leaf area; reduced oxygen supply to roots and a mechanical resistance to vertical growth (Yu *et al.*, 2002; Maun, 2004; Dech & Maun, 2006; Li *et al.*, 2010). There are several responses by which plants can mitigate these effects including rapid shoot elongation (Voisenek *et al.*, 1998; Yu *et al.*, 2002; Dech & Maun, 2006; Gilbert & Ripley, 2008; Jia *et al.*, 2008; Liu *et al.*, 2008), production of adventitious roots (Dech & Maun, 2006) and resource reallocation and remobilization (Yu *et al.*, 2004; Gilbert & Ripley, 2008). Shoot elongation appears to be a particularly common response (Dech & Maun 2006), and is believed to be beneficial as it enables plants to position their leaf area above the rising soil level. Thus a strong burial-induced shoot elongation response may confer an advantage in terms of increased survival in habitats with moving sands (Maun, 2004).

Tolerance to burial has been found to differ considerably among species, and burial has been considered to be an important selective force (Brown, 1997; Maun, 1998; Dech & Maun, 2006; Liu *et al.*, 2008). It is also known that the extent of sand movement depends on many factors, such as local vegetation cover, wind exposure, activated surface of dune associated with wind direction, soil texture and moisture (Maun, 1998; Yu *et al.*, 2004), all of which can vary considerably in time and space. As a result there is often strong small-scale spatial variation in the degree of sand movement and associated burial and denudation of plants (Maestre & Reynolds, 2006; Housman *et al.*, 2007; Okayasu *et al.*, 2012). The presence of spatial variation in burial risk and extent could lead to genetic variation in resistance to burial. While intra-specific variation in plasticity in stem traits in response to other environmental factors such as canopy shading (shade avoidance; Botto & Smith, 2002; Huber *et al.*, 2004; Weijschedé *et al.*, 2006, 2008) and flooding (Chen *et al.*, 2009, 2011; Huber *et al.*, 2009) have been well documented, such variation in responses to sand burial has been rarely investigated.

Phenotypic plasticity in plants is often investigated with respect to responses in individual traits, especially leaf and stem traits (Cipollini & Schultz, 1999; Botto & Smith, 2002; Huber *et al.*, 2004, 2009, 2012;

Weijschedé *et al.* 2006, 2008; Chen *et al.*, 2009, 2011). Plants like all organisms are highly integrated systems where the functioning of one trait is strongly dependent on other traits (Lenssen *et al.*, 2004; Weijschedé *et al.*, 2006, 2008; Huber *et al.*, 2012). Therefore, functionally coordinated plasticity of several traits would be expected. As noted above there is a clear trade-off between plant height and mechanical stability (Niklas, *et al.*, 2006; Vospernik *et al.*, 2010). Increased stem elongation will make plants more prone to mechanical damage, either through excessive self loading or due to external forces such as wind drag (Niklas, 1992; Anten *et al.*, 2005). In drylands areas the risk of mechanical damage might be especially large due to the general prevalence of high wind speeds. However, this risk can be mitigated if increased stem elongation is associated with increases in stem diameter and stem tissue stiffness and strength (Goodman & Ennos, 1996). From this perspective plasticity in stem elongation would be expected to be functionally coordinated with plasticity in stem diameter growth and/or stem tissue mechanical properties.

Still, under resource limitation, investment in one function (e.g. height growth) could be expected to go at the expense of other functions (e.g. stability; Cipollini & Schultz, 1999). Drylands ecosystems are characterized by low water availability, imposing important constraints on growth, and thus probably investment in one trait would go at the cost of another. Such a trade-off may constrain the expression of the optimal response of individual traits.

Seedlings of 18 different seed families of a common shrub species in Mu Us Sandland, *Caragana intermedia*, were used in this experiment. Plants were exposed to two sequential burial events in which each time two thirds of the aboveground height was covered with sand. We addressed the following questions: (1) What is the impact of burial on stem traits including stem elongation, diameter growth and tissue strength and stiffness? (2) To what extent is there intra-specific variation in these traits? (3) To what extent is there intra-specific variation in plastic changes of stem traits in response to burial?

Material and methods

Focal species and study site

Caragana intermedia Kuanget (Fabaceae), a deciduous shrub species used to fix dunes and reduce wind speed, is widely distributed in the Inner Mongolia Autonomous Region, Ningxia Autonomous Region and Shanxi Province of

China (Xiao *et al.*, 2003). It typically grows to a height of 70–150 cm and has compound and plumose leaves (Fu, 1993). The species can endure cold, drought, sand burial and high levels of wind and can grow in regions with an annual rainfall as low as 300 mm. It also occurs widely and commonly along a gradient of sand mobility, from mobile to fixed dunes (Fu, 1993; Guo *et al.*, 2010). That makes the species an ideal model system to study intra-specific variation in burial responses. It is also an important economic crop used as fodder (Guo *et al.*, 2010).

In July of 2010, we collected seeds of *Caragana intermedia* from 18 mother plants around Ordos Sandland Ecological Research Station (OSES, 39°29'37.6"N, 110°11'29.4"E, 1300 m a.s.l.) of the Institute of Botany, the Chinese Academy of Science, located in Mu Us Sandland in Inner Mongolia, China. The average summer temperature is 20–24 °C and average annual precipitation is 260–450 mm concentrated from July to September (Zhang, 1994). Mother plants were distributed within an area of approximately 3 km². Seeds collected from different plant individuals were considered to be separate seed families. The experiment was conducted in the plastic greenhouse compartment located at the Utrecht University Botanical Gardens, the Netherlands (52° 5' 16.79"N, 5° 10' 8.26"E) in the summer of 2011.

The experiment and measurements

On 19 April 2011, seeds were placed on the surface of shallow plastic square boxes (30 cm×15 cm×5 cm, 2.25L) filled with sand for germination. On 26 April 2011, germinated seeds were transplanted to small square pots (7 cm×7 cm×8 cm, effective volume 0.24 L). From 7 June to 10 June 2011, seedlings of each group at similar height from 4 cm to 6 cm were selected and transplanted to large square pots (15 cm×15 cm×20 cm, effective volume 3.5 L) filled with sand and two grams of solid slow-release fertilizer (16N-11P₂O₅-11K₂O-3MgO + Te, 3-4 months, Osmocote Exact, Scotts International B.V, Heerlen, Netherlands). As a result of the different number of available seeds per seed family, germination rate and growth rate, numbers of seedlings of each seed family varied from 10 to 30 (Table 1 in Appendix). In total there were 412 plants for the experiment.

From 16 to 18 June 2011, we randomly chose half of the plants from each seed family and buried them with sand up to two thirds of their height. A transparent plastic bag was used to wrap the plant to hold the sand. Four thin sticks were inserted into the sand to support the bag. Positions of plants were randomly arranged at the beginning and changed every week. Initial height was measured to determine the burial level and shown in Table 1.

Immediately after the first burial, height and basal diameter above soil surface were measured (first measurement, H_1 and D_1 ; see Fig. 1a describing the experimental set up and timeline). For unburied plants we also measured the diameter at two thirds height which was developmentally similar to the basal part of buried plants (see Fig. 1b). Hereafter this measure was called intermediate diameter and abbreviated as ID. In this way, we could test the burial effect on diameter growth by comparing developmentally similar sections of buried and unburied plants. We used the actual height above the soil level to calculate the height growth rate and mechanical traits. From 5 July to 7 July 2011, height, basal and intermediate diameter were measured again (second measurement, H_2 , D_2 and ID_2).

From 25 July to 27 July 2011, we repeated the burial treatment whereby shoots were buried again up to $2/3$ of their height above the soil. Height and basal diameter were measured before and after the treatment (third measurement, H_{3-1} , D_{3-1} and ID_3 before burial and H_{3-2} and D_{3-2} after burial). Intermediate diameter was only measured before the second burial (i.e., at H_1 , H_2 and H_{3-1}). Seventeen days later, height and basal diameter were measured to calculate the growth characteristics (fourth measurement, H_4 and D_4).

The final harvest was conducted from 1 to 7 September 2011. We measured aboveground height and basal diameter in the greenhouse (fifth measurement, H_5 and D_5). Harvested plants were packed in wet tissue to avoid turgor loss and taken to the lab. Plants were separated into leaves, stems and roots with a sharp scissors. Roots were carefully washed. Three mechanical traits of stems, including Young's modulus (E , a measure that indicates tissue stiffness), breaking stress (σ_b , a measure indicating tissue strength) and maximum load force of main stems, were measured applying the three-point bending technique (for details see Anten *et al.*, 2009) with a universal electromechanical testing machine (Type 5542, Instron, Norwood, Massachusetts, USA). Vertically applied forces (F ; N) and resulting deflections (δ ; m) were recorded. The distance between supports was adjusted such that it was always at least ten times larger than the diameter of the stem section. The dry biomass was determined after the roots, stems and leaves were dried in a stove at 70°C for 48 hours.

Data processing

Mean absolute growth rates of height (G_H) and basal diameter (G_D) during the four stages were calculated with the data of five measurements: e.g. for stage 1 from census 1 to 2 as $(X_2 - X_1)/t_{12}$ with X the trait value measured at each census and t the time between the censuses (see also Fig. 1a). Intermediate

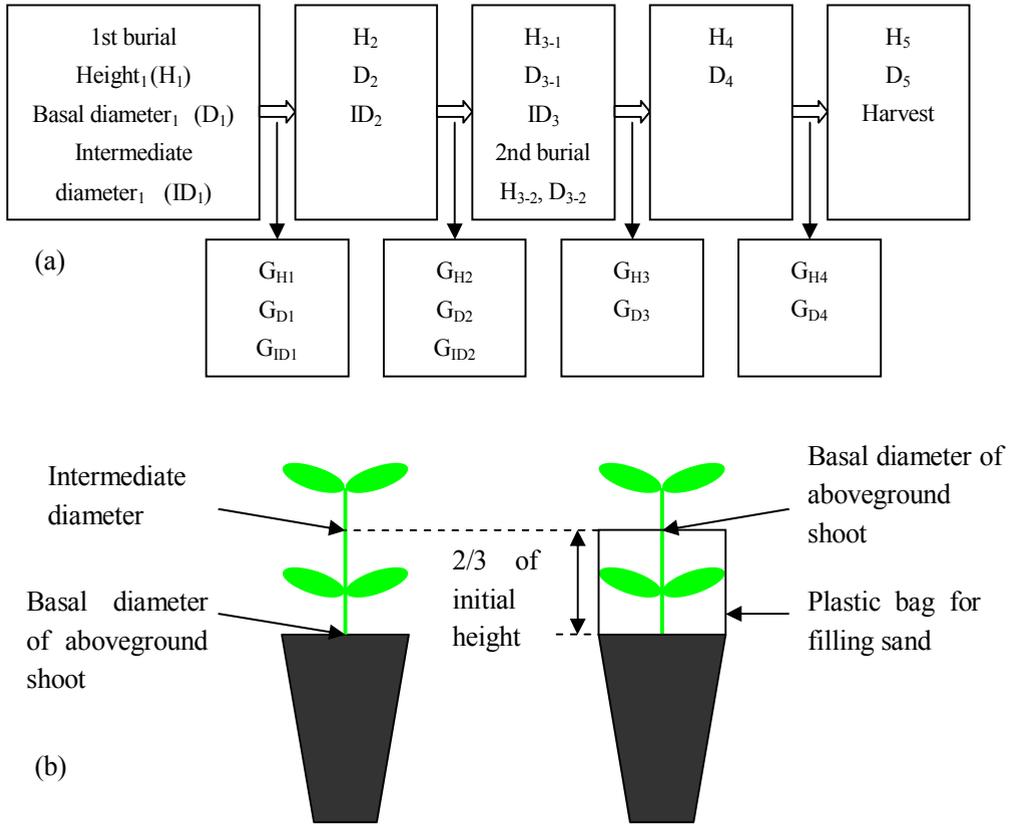


Fig. 1. Schematic illustration describing the process of the whole experiment (a) and the definition of basal diameter and intermediate diameter (b). At both burial events, two thirds of shoot height above soil surface were buried by sand. Height measurement refers to aboveground shoots. Intermediate diameter was only measured twice between first and second treatments. At the third measurement, height and basal diameter were measured twice, before and after burial. H_{3-1} and D_{3-1} before burial were used for calculation of G_{H2} and G_{D2} and H_{3-2} and D_{3-2} after burial were used for calculation of G_{H3} and G_{D3} . Basal diameter refers to the basal stem section above the soil surface for both buried and unburied plants. Intermediate diameter is the section at two thirds of initial height of unburied plant, and is relatively at the same position as the basal part of buried plant between first and second treatment. For buried plants, height above the soil surface plus height of stem in burial were used for calculation of plasticity.

diameter growth (G_{ID}) was calculated in the same manner, but only for the two periods between the first and second burial (note that for buried plants $G_D = G_{ID}$ but that for unburied plants $G_D \neq G_{ID}$, see Fig. 1). Mechanical traits, stem tissue stiffness (Young's modulus, MPa, E in short hereafter; Gere & Timoshenko, 1999; Anten *et al.*, 2005), geometric contribution of basal section of stem to stiffness (second moment of area, mm^4 , I in short hereafter; Jaffe *et al.*, 1984; Niklas, 1996), rigidity of basal cross section (flexural stiffness, N m^2 , EI in short hereafter) and stem breaking stress (σ_b , MPa) were measured to determine the mechanical stability of the stems. Details of calculations of these mechanical traits can be found in Anten *et al.* (2005).

The plasticity of plants in response to burial were calculated as follows:

$$\text{Plasticity} = (\text{VALUE}_{\text{buried}} - \text{VALUE}_{\text{unburied}}) / \text{VALUE}_{\text{unburied}} \quad (1)$$

whereby $\text{VALUE}_{\text{buried}}$ and $\text{VALUE}_{\text{unburied}}$ refer to trait measures (e.g. height, diameter or mechanical traits) of individual buried and unburied plants, respectively. This equation considers plasticity in relative rather than absolute terms as it calculates the percentage change in length in buried as compared to unburied plants (see Weijsschedé *et al.* 2006). Positive values of plasticity indicate larger trait values under buried than under unburied conditions. Strongly positive or negative plasticity values indicate a large degree of plasticity. Calculations were done within seed family. In contrast to Weijsschedé *et al.* (2006) plasticity was not calculated using genotypic means but plants within seed family were paired prior to calculating plasticity in order to get independent measures of plasticity within seed family which allows for statistical comparison of plasticity of the different seed families. Within family pairing was done according to H_1 (i.e., the tallest unburied plant at H_1 was coupled with the tallest buried plant, etcetera) in order to take account of different initial height of plants. It should be noted that plasticity calculations of height were based on total height, thus including the stem section below the soil in buried plants. The reason is that because aboveground height is initially reduced by burial, plasticity value become misleading when based on aboveground height rather than total height. For example, a plasticity value of zero with aboveground height suggests there not no have been plastic response to burial, while in reality buried plant had caught up with unburied ones. For plasticity of diameter, we used basal diameter of buried plants and intermediate diameter of unburied between first and second treatments (Fig. 1). As noted above this ensured that we compared developmentally similar stem sections.

Data analysis

Two-way ANOVA was used to test for the effects of burial and seed family on growth, morphological and mechanical traits. Burial was set as a fixed factor while seed family was set as random factor. A one-way ANOVA was used to test the differences in plasticity among seed families. Before analysis, data were checked for equality of variance with Levene's test and for normality with Shapiro-Wilk test. Subsequently, all data were Ln-transformed. The regression equations in the figures were generated by Sigmaplot 11.0 (Systat Software Inc., Erkrath, Germany). An ANCOVA was used to test whether the regression lines of final height (covariate) with other traits (dependent variable) differed between burial treatments. SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA) was used for all statistical analyses. Here we chose $P < 0.05$ as significance level.

Results

Burial affected almost all of the measured traits. (Table 1; Figs. 2, 3, 4, 5). Burial had a positive effect on stem elongation rates calculated for each of the four growth periods except for the second period (Table 1; Fig 3a, d). This stimulation in stem elongation did not compensate for the initial reduction in aboveground height caused by the burial treatment itself, so that height above the soil was consistently lower in buried plants (Table 1; Fig. 2, only data for fifth census H_5 are shown). Generally buried plants exhibited faster diameter growth than unburied plants when comparing stem sections of the same physiological age ($G_{D\text{-burial}}$ vs. G_{ID} at 1st and 2nd stages; Table 1; Fig. 3c, f), but slower growth when comparing basal stem sections ($G_{D\text{-burial}}$ vs. $G_{D\text{-unburial}}$) except for the first stage (Table 1; Fig. 3b, e). In combination with the effect of stem tapering this caused buried plants to have lower basal diameters resulting in lower second moments of area (I ; Table 1; Fig 2c). Burial did result in an increase in stem tissue stiffness (Young's modulus, E ; Table 2; Figs. 2b, 4a) but this was insufficient to compensate for the lower I such that the flexural stiffness (EI) of the basal stem section was lower in buried than in unburied plants (Table 2; Figs. 2e; 4c). Finally burial resulted in more allocation of mass to stems and leaves at the expense of roots (Table 2; Fig. 5), but total plant mass was smaller in buried than in unburied plants (Table 2; Fig. 2f).

Plants from different seed families differed significantly with respect to many traits, e.g. height, basal diameter, height and diameter growth rate, the fractional allocation of mass to stems, leaves and roots, and I (Table 2; Figs. 2, 3, 4, 5). Significant interactive effects of burial and seed family were found for

E , I and EI (Table 1). Particularly the direction of the burial differed between seed families; most seed families exhibited increases E and reductions in I and EI , but a few seed families showed the opposite responses (Figs. 4a, b, c). There was also a significant difference in plasticity in these mechanical characteristics among seed families but this difference was not significant for any of the other traits (Table 2; Fig. 4). Interestingly the plasticities in all mechanical traits were positively correlated with the plasticity in final height (Fig. 4).

Table 1. Two-way ANOVA for test effects of burial (B) and seed family (SF) on growth (G), morphological (H: height; D: diameter, for explanation of abbreviations used to indicate the different height and diameter measurements see Figure 1) and mechanical traits.

Morphology									
	H ₅	D ₅	G _{H1}	G _{H2}	G _{H3}	G _{H4}	G _{D1}	G _{D2}	G _{D3}
B	62.12 ^{***}	292.43 ^{***}	42.18 ^{***}	0.00 ^{ns}	8.89 ^{**}	6.98 [*]	0.59 ^{ns}	41.55 ^{***}	137.20 ^{***}
SF	3.30 ^{**}	1.29 ^{ns}	2.80 [*]	3.68 ^{**}	4.04 ^{**}	5.74 ^{***}	1.40 ^{ns}	1.26 ^{ns}	3.92 ^{**}
B×SF	1.07 ^{ns}	1.75 [*]	0.93 ^{ns}	0.80 ^{ns}	0.79 ^{ns}	0.71 ^{ns}	0.91 ^{ns}	1.16 ^{ns}	0.40 ^{ns}
	G _{D4}	G _{ID1}	G _{ID2}						
B	12.61 ^{**}	6.53 [*]	4.67 [*]						
SF	1.66 ^{ns}	1.00 ^{ns}	3.11 [*]						
B×SF	1.47 ^{ns}	1.33 ^{ns}	0.66 ^{ns}						
Growth									
	Total Mass		Leaf mass fraction		Stem mass fraction		Root mass fraction		
B	40.40 ^{***}		20.12 ^{***}		125.08 ^{***}		192.62 ^{***}		
SF	1.74 ^{ns}		2.33 [*]		6.14 ^{***}		2.87 [*]		
B×SF	1.41 ^{ns}		1.41 ^{ns}		1.02 ^{ns}		1.09 ^{ns}		
Mechanical trait									
	Young's modulus		Second moment of area		Flexural stiffness		Breaking stress		
B	0.40 ^{ns}		294.46 ^{***}		193.94 ^{***}		4.69 [*]		
SF	1.33 ^{ns}		1.30 ^{ns}		1.42 ^{ns}		2.38 [*]		
B×SF	1.98 [*]		1.73 [*]		1.79 [*]		1.32 ^{ns}		

H₅ and D₅ here are the final height and basal diameter. Growth rate of height and diameter of four stages are shown. G_{ID} presents comparison between the basal section of buried plants and the intermediate section of unburied plants in the first two stages. F values and the significance levels (^{***} $P < 0.001$, ^{**} $P < 0.01$, ^{*} $P < 0.05$ and ^{ns} $P \geq 0.05$) are given. Data were Ln-transformed before analyses.

Table 2. One-way ANOVA for test of plasticity of morphological, growth and mechanical traits.

	H ₂	D-ID ₂	H ₃₋₁	D-ID ₃	H ₄	H ₅	Total mass
Seed family	2.06*	0.87 ^{ns}	1.12 ^{ns}	0.97 ^{ns}	0.95 ^{ns}	0.76 ^{ns}	1.20 ^{ns}
	Young's modulus	Second moment of area	moment	Flexural stiffness		Breaking stress	
Seed family	1.97*	1.77*		1.76*		1.53 ^{ns}	

For plasticity of height, the height above soil surface plus the height of stem in burial were used. For the data of height and diameter of third measurement, values before the second burial treatment were used. Test for diameter was only conducted between basal section of buried plant and intermediate section of unburied plant at same developmental ages was conducted at first and second stages. *F* values and the significance levels (* $P < 0.05$ and ^{ns} $P \geq 0.05$) are given. Data were Ln-transformed before analyses.

Table 3. ANCOVA test for differences of regression lines in burial treatment of Fig. 2.

	D ₅	Young's modulus	Second moment of area	Breaking stress	Flexural stiffness	Total mass
H ₅	53.53**	17.71***	38.87***	35.44***	60.24***	73.91***
Burial	109.76**	13.22**	103.96***	6.64*	72.20***	0.02 ^{ns}

Mean values of 18 seed families were used for ANCOVA test. Burial was treated as fixed factor in all the analyses. H₅ was treated as covariate and other traits were treated as dependent variable. *F* values and the significance levels (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ and ^{ns} $P \geq 0.05$) are given. Significance of H₅ with other trait means that there is correlation between them. Data were Ln-transformed before analyses.

In general, the effects of burial on stem elongation changed over time. The first burial event induced on average a 55% increase in shoot elongation (G_{H1}) but the stimulatory effect did not persist (G_{H2}). The second burial induced only a 10% increase in stem elongation (G_{H3}) and this effect persisted (G_{H4}; Table 2; Fig. 3a, d).

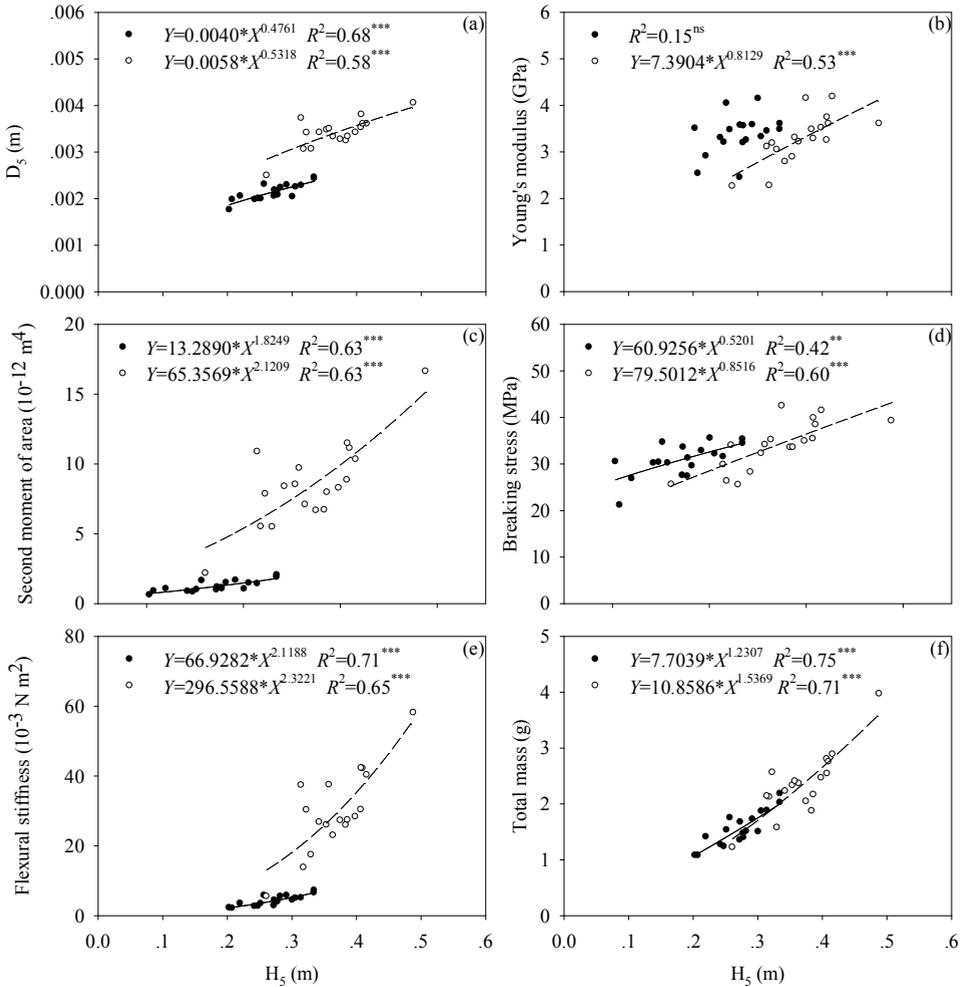


Fig. 2. Relationship between final aboveground height (H_5) and basal diameter (a), Young's modulus (b), second moment of area (c), breaking stress (d), flexural stiffness (e) and total mass (f) of buried (●;—) and unburied (○;---) plants of 18 seed families. Each point indicates mean values for seed families per burial treatment. Regression equations, coefficient of determination (R^2) and significance level ($^{***}P < 0.001$, $^{**}P < 0.01$ and $^{ns}P \geq 0.05$) are given. Results of ANCOVA testing whether the regression lines differ between burial treatments are shown in Table 3.

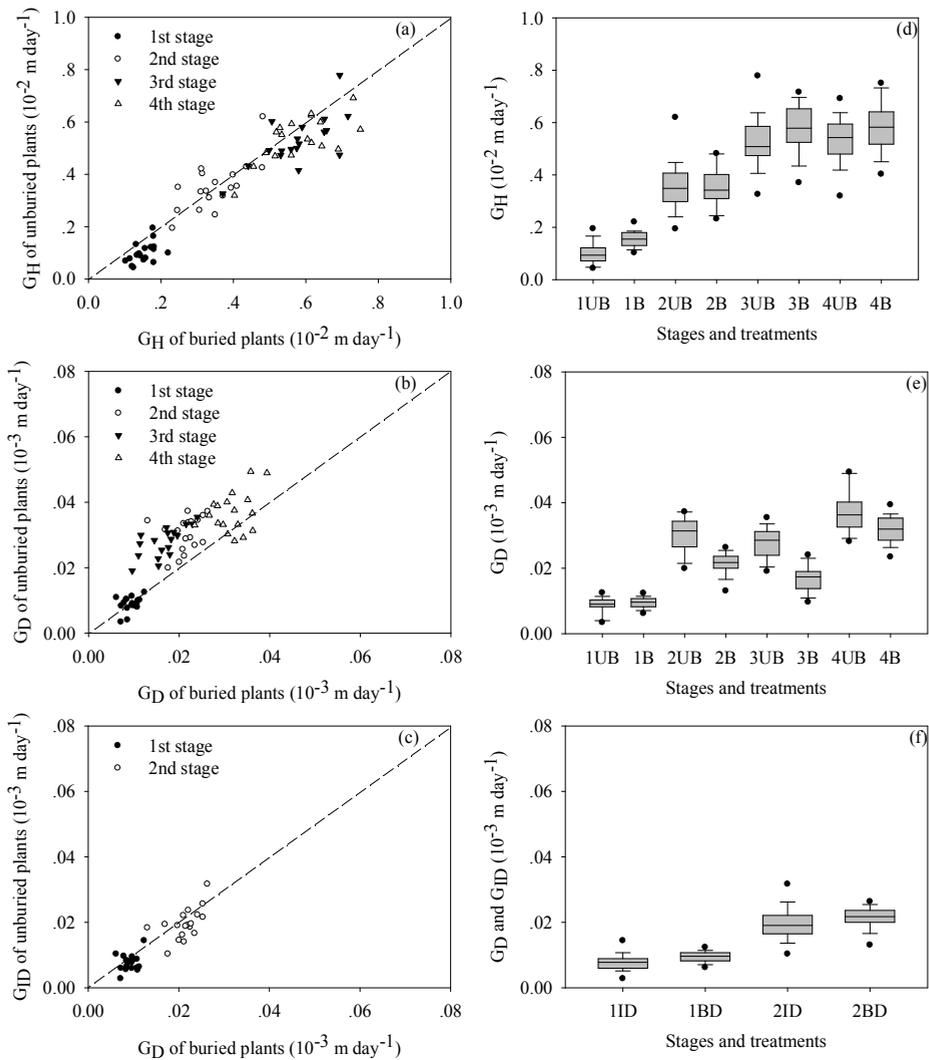


Fig. 3. Growth rate of height (G_H ; a) and basal diameter (G_D ; b) of buried and unburied plants of 18 seed families across four stages, and growth rate of intermediate diameter (G_{ID} ; c) of unburied plants of 18 seed families at first and second stages. The broken line (---) represents $y = x$. The box-plot graphs show the variation in G_H (d) and G_D (e) of buried (B) and unburied plants (UB) across four stages and in G_D of buried plants (BD) and G_{ID} of unburied plants (ID; f) at first and second stages, with seed families being lumped together.

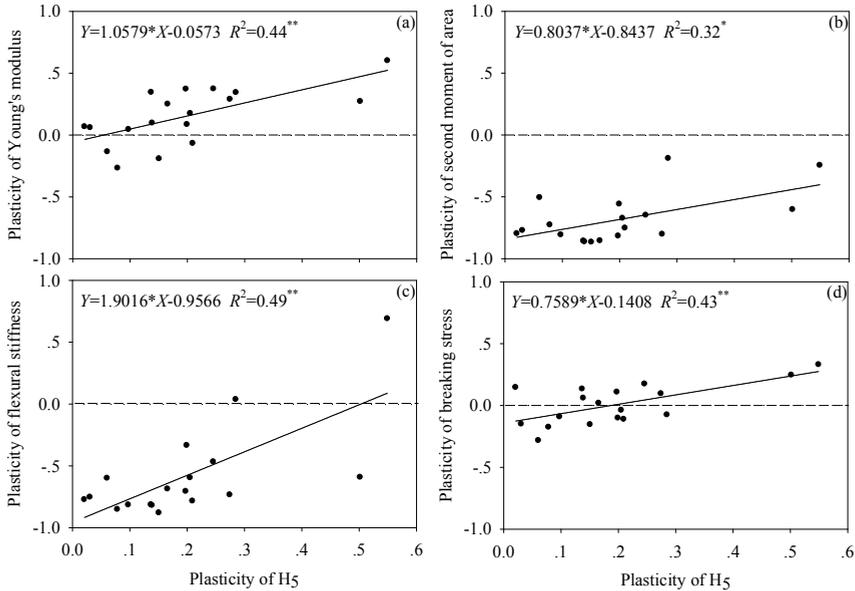


Fig. 4. Relationship between plasticity of final aboveground height and plasticity of Young's modulus (a), second moment of area (b), flexural stiffness (c) and breaking stress (d) of 18 seed families. Regression equations (—), coefficient of determination (R^2) and significance level (** $P < 0.01$ and * $P < 0.05$) are given. The broken line (---) represents $y = 0$.

In terms of allometric growth patterns, variation in stem height across seed families was positively correlated with variation in basal diameter (growth), E , I , breaking stress (σ_b), EI and total mass (Fig. 2). However there was a clear effect of burial on this pattern. The scaling coefficient values of b in the allometric relationship between final height (H_5) and basal diameter (D_5 ; $H_5 = aD_5^b$; the other traits are same), I , σ_b , EI and total mass were lower in buried plants. As a result of differential responses of E among seed families to burial, height of buried plants was not correlated with E while the height of unburied plants was (Fig. 2b).

Discussion

Burial effect on stem elongation

In our study sand burial significantly stimulated stem elongation, consistent with several other studies (Voesenek *et al.*, 1998; Dech & Maun, 2006; Gilbert & Ripley, 2008; Jia *et al.*, 2008; Liu *et al.*, 2008). A direct effect of burial is loss of plant photosynthesis as leaves are being covered and are thus

no longer exposed to light. Increase in stem elongation is thus believed to entail an important compensatory response as it enables plants to keep leaves above the rising soil level. The physiological mechanism underlying this response is not fully clear, but a role of ethylene accumulation has been suggested. Ethylene has been documented to play a role in the stimulation of stem elongation in response to both the presence of neighbor plants (Pierik *et al.*, 2004) and flooding (Voesenek & Blom, 1998). Burial results in mechanical impedance to plants, which possibly increases the production rate of ethylene as a result of the relatively large resistance of soil to air diffusion (Voesenek *et al.*, 1998). Indeed ethylene was implicated to be involved in burial induced stimulation of stem node formation but not in stem elongation. Other factors that could play a role are shading (burial places part of the plant in almost complete darkness) and CO₂ accumulation but no clear evidence exists for their roles (Voesenek *et al.*, 1998).

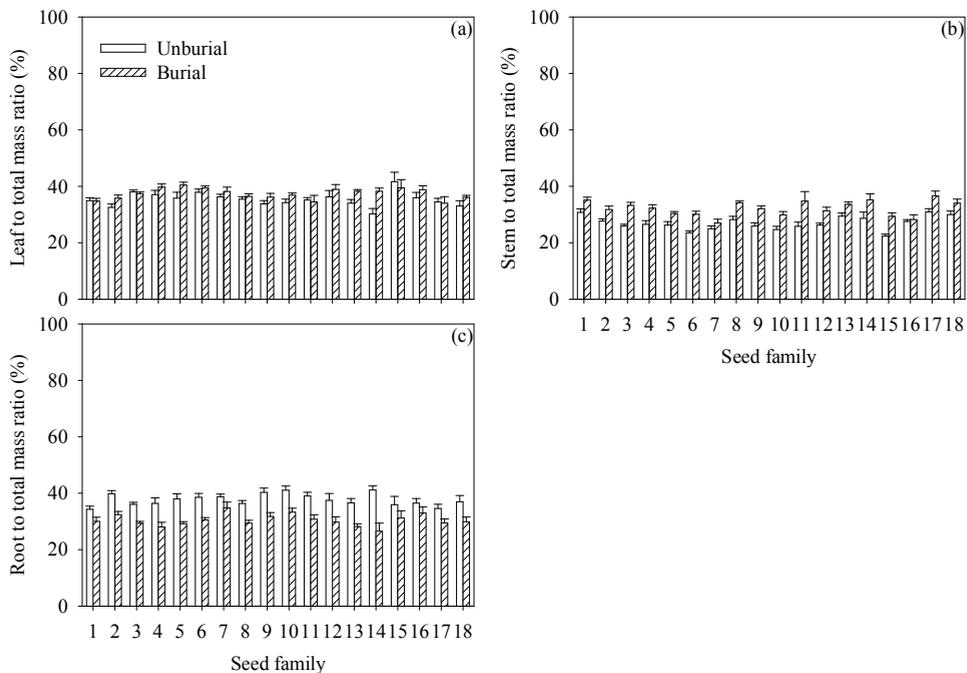


Fig. 5. Ratio of leaf (a), stem (b) and root (c) mass to total mass of 18 seed families in burial or not.

Responses of shoot elongation to burial appeared to depend on the developmental stage of plants. Just after the first burial, elongation rate (G_{H1}) of buried plants was on average about 55% larger than that of the unburied ones, while the second burial treatment had a much smaller stimulatory effect (about 10% increase in stem elongation (G_{H3} and G_{H4}). It has been argued that plastic responses to environmental changes early in life may constrain responses to the same cue later on (Sultan, 2000; Weinig & Delph, 2001; Huber *et al.*, 2012). For example Weinig & Delph (2001) reported that *Abutilon theophrasti* seedlings that had been exposed to a prior spectral shading treatment were less responsive to a subsequent treatment than seedlings that had not received the first treatment. They argued that the earlier responses entailed costs in terms of resource or reduced mechanical stability that limited later responses. More recent work indicates that the extent to which early responses affect responses later in life is modulated by the internal nutrient status of the plant (Huber *et al.*, 2012).

Elongation and other correlated traits

As noted, elongation without a concomitant increase in stem diameter and/or stem tissue stiffness and strength may entail a mechanical risk. It should be noted in this respect that just after implementation of burial treatments height above the soil was three times smaller in buried plants, but that due to stem tapering the second moment of area (i.e. the geometric contribution of basal area to stiffness) just after the two burial treatments were 81% and 63% smaller, respectively. Thus a response in radial growth or tissue properties that is functionally coordinated with stem elongation would be expected. Indeed we observed a stimulatory effect of burial on stem radial growth, at least when comparing stem sections of the same developmental stage (see G_{ID} in Table 1 and Fig. 3c, f), indicating that plasticity in stem elongation was at least to some extent functionally coordinated with an increase in diameter growth (see Fig. 1). This result is contrary to studies on shade avoidance that found that shading stimulated stem elongation but inhibited diameter growth (Kennedy *et al.*, 2007; Anten *et al.*, 2009). This lack of mechanically coordinated response in stem length and diameter growth under shading could be associated with the low resource availability in a shaded environment (typically shading experiments reduce light levels by more than 70%).

Burial increased both tissue stiffness (Young's modulus, E) and strength (breaking stress, σ_b) of the basal stem section. A possible explanation for this effect could be that the basal stem section of buried plants was younger and that the composition younger tissue may be more easily changed under

influence of external cues (Speck & Rowe, 1999; Isnard *et al.*, 2003). Unfortunately we did not measure the tissue mechanical traits on intermediate stem sections on unburied plant that were at a similar developmental stage as the basal sections of the buried plants. Together however these results indicate that burial-induced increases in stem elongation were coordinated with similar increases in stem diameter, as well as tissue strength and stiffness.

Allometric growth of height and basal stem diameter is especially important to explore functional biomechanics as this relationship strongly determines the ability of a structure to support its own weight (Niklas *et al.*, 2006). In this respect it is again important to note that while burial stimulated diameter growth when comparing stem sections of the same age, the combination of stem tapering and burial caused diameters at ground level to be smaller in buried than in unburied plants. The consequence was that buried plants had stems with smaller basal diameters and associated lower flexural rigidity at a given stem height than unburied plants. Apparently even though burial stimulated stem radial growth, this effect was insufficient to compensate in mechanical terms for increase in stem length, which possibly will lead to a risk of mechanical rupture.

Difference among seed families

Seed families differed from each other in a number of traits e.g. height, diameter, growth rates of height and diameter, leaf characteristics, total mass and a series of mechanical traits. Especially, there was an almost two-fold variation in mean height growth among seed families. This suggests that there was a strong genetic variation in stem growth and mechanical traits among individuals even within a relatively small area (3 km²).

The effects of burial on stem mechanical traits (Young's modulus, flexural stiffness and breaking stress) differed significantly among seed families. This was indicated both by the significant seed family \times burial interaction on these traits as well the significant difference between seed families in their calculated plasticity. While intra-specific variation in plasticity in stem traits to other cues such as shading (e.g. Dudley & Schmitt, 1995; Botto & Schmitt, 2002; Anten *et al.*, 2009) and flooding (Voesenek & Blom, 1998; Huber *et al.*, 2012) have been documented, our study is the first to show such variation in plasticity in response to burial. Some variation in the response to sand burial would be expected within *C. intermedia* as it commonly occurs along a gradient of sand mobility (Fu, 1993; Guo *et al.*, 2010). The mean plasticity indices for height also differed considerably between seed families, i.e., ranging from almost no response to a three-fold increase in height growth.

However, due to the large variation in data this pattern was not significant. It is nevertheless interesting to note that the plasticity values for tissue traits correlated positively with the plasticity values for height growth. That is, the seed families that exhibited relatively strong increases in tissue strength and rigidity were also those that tended to have the largest response in height growth. This suggests that variation in plasticity in different traits in response to burial is functionally coordinated with respect to mechanical stability (Botto & Smith, 2002).

This result appears to be contrary to similar studies on shade avoidance. Anten *et al.* (2009) documented intra-specific variation caused by shade avoidance in stem traits (stem elongation, Young's modulus and stem diameter), but the variation in these traits was not correlated. As a result of this apparent lack of coordination in the plasticity in stem traits genotypes with a larger degree of shade-induced stem elongation exhibited reduced mechanical stability up to the point that the most plastic genotypes in terms of stem elongation became unstable under shading. Botto & Smith (2002) also documented strong intra-specific variations in flowering acceleration and elongation in response to canopy shading across more than 100 accessions of *Arabidopsis thaliana* but these responses were not correlated. This lack of correlation has been argued to indicate that microevolution in the pathways downstream from signal perception has occurred separately (Botto & Smith, 2002). It could thus similarly be argued that apparent correlation between burial-induced changes in stem elongation and changes in stem mechanical traits observed by us indicates a coordinated microevolution. While this presents an interesting perspective on the plasticity in functionally coordinated traits, we stress that more research is needed.

Conclusions

Burial-induced plastic responses in stem elongation differ widely between species and have been clearly associated with burial tolerance (Brown, 1997; Maun, 1998; Dech & Maun, 2006; Liu *et al.*, 2008). For *Caragana intermedia* we showed that burial-induced elongation was associated with a concomitant increases in stem radial growth and tissue stiffness and strength, but burial nevertheless caused a shift in the allometric stem growth trajectory towards greater stem slenderness. In addition, we are the first to document that there is a strong intra-specific variation in the plastic responses to burial in stem mechanical traits, which appears to be functionally coordinated the variation in the responses in stem elongation. More generally our results emphasize the

importance of considering functionally coordinated traits when analyzing the consequences in phenotypic plasticity in plants.

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Appendix

Table 1. Numbers of replicates and mean initial height of each seed families at beginning of the experiment.

Seed family	Number	Initial height (cm)
1	30	6.84±0.15
2	28	7.11±0.13
3	24	7.15±0.17
4	26	7.19±0.18
5	16	6.84±0.16
6	28	7.16±0.18
7	28	6.81±0.17
8	30	6.87±0.16
9	28	7.64±0.15
10	24	6.94±0.17
11	22	6.56±0.17
12	22	7.06±0.18
13	30	7.40±0.12
14	12	6.57±0.19
15	10	4.63±0.30
16	12	5.92±0.32
17	28	7.27±0.18
18	14	5.64±0.31

Data are means ± SE



One of mother plants of *Caragana intermedia*

Chapter 6

General discussion and summary

This thesis reports on the plastic response of dominant woody species in Mu Us Sandland, Inner Mongolia, China, to local stress e.g. drought, grazing, sand burial, mechanical stress (wind load and trampling) and the interactive effects of these factors. In addition, the role of physiological integration in helping clonally growing plants to mitigate the negative impacts of these stress factors was investigated. This dissertation did not only compare the phenotypic plasticity among different species, but also analyzed the intra-specific variation in plasticity. In this chapter, I briefly discuss the variation in resistance and adaptation to the stress factors mentioned above among plants. I will also analyze the degree to which responses in different traits to a given stress factor are functionally coordinated. Finally I discuss the significance of the results of this study in the context of management and restoration of vegetation in drylands ecosystems. A schematic overview of the main results and their connotations is presented in Fig. 1

1. Sand burial

In drylands ecosystems vegetation degradation resulting from e.g. overgrazing and/or climate change, causes increased exposure of bare soil which in combination with prevalently high wind speeds increases the probability of sand movement. This in turn may cause plants to be buried and this impacts plants: coverage of photosynthetic leaf area, increased resistance to upward growth, and reduced oxygen availability to roots. Deep and/or long-standing burial can be fatal to plants (Maun, 2004). Tolerance to burial has been found to differ considerably among species (Brown, 1997; Maun, 1998; Dech & Maun, 2006; Liu *et al.*, 2008). As one of the main responses to burial, increased aboveground height of stem is thought to be important for survival and growth as it enables plants to maintain leaves above the soil (Voesenek *et al.*, 1998; Dech & Maun, 2006; Gilbert & Ripley, 2008; Jia *et al.*, 2008; Liu *et al.*, 2008). Besides, burial tolerance in woody plants also involves other adaptive traits e.g. production of adventitious roots, increases in photosynthetic capacity of the leaves remain above the soil, and resource remobilization (Dech & Maun, 2006; Perumal & Maun, 2006; Gilbert & Ripley, 2008). In this thesis I primarily focused on burial-induced stem

elongation.

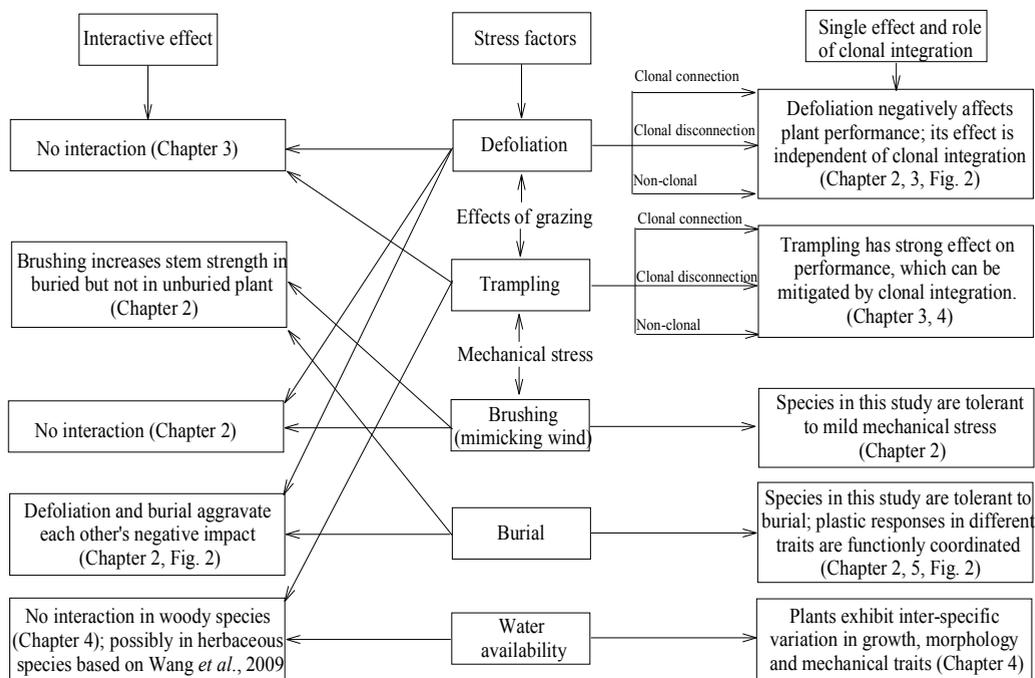


Fig. 1. A schematic representation of the singular and interactive effects of the dominant stress factors on plants in drylands ecosystems including the role of clonal integration as studied in this thesis. Combination of three stress factors are also studied in Chapter 2 (brushing × burial × defoliation), Chapter 3 (clonal integration × defoliation × trampling) and Chapter 4 (species × trampling × water availability) to understand the complicated interaction.

Physiological and time-dependent regulation of burial-induced stem elongation

As mentioned above, increase in aboveground height is an important and common response to burial. Increased shoot length after burial was also found among different species in this thesis (*Cynanchum komarovii* in Chapter 2; *Caragana intermedia* in Chapter 5; *Artemisia ordosica* in Fig. 2 in current Chapter) to different degrees. Furthermore, speed of shoot elongation raised with increasing gradient of burial levels (Fig. 2 in current Chapter).

The physiological mechanism regulating the burial-induced elongation response is not well understood, but a role of ethylene accumulation has been suggested (Voesenek & Blom, 1998; Voesenek *et al.*, 1998). Ethylene has been shown to be involved in shade-induced elongation (Pierik *et al.*, 2004). Mechanical stress on plants may stimulate ethylene formation while sand

imposes a relative resistance to gas diffusion, so that ethylene accumulation under burial is likely to occur. It is also suggested that ethylene is involved in burial-induced stimulation of stem node formation but not in internode elongation (Voeselek *et al.*, 1998). Other factors that could be effective are shading (burial induces almost complete darkness to part of the plant) and CO₂ accumulation but no clear evidence exists for their roles (Voeselek *et al.*, 1998).

My results indicate that the effects of burial on stem elongation may depend on the developmental stage of plants and/or their burial history. In Chapter 5, the first burial treatment applied to seedlings of *C. intermedia*, which were about two months old and on average 6.9 cm tall, had very strong effects on stem elongation (about 53% increase in stem elongation as compared to unburied plants). Interestingly the second burial treatment, applied about one month after the first, had a much smaller stimulatory effect (about 10% increase in stem elongation). This pattern could be associated with a limited storage of reserves in plants. Burial induced elongation probably involves the reallocation and mobilization of resources from buried leaves (Freas & Kemp, 1983; Gilbert & Ripley, 2008), which may become depleted after repeated burial. Similar patterns have been observed with defoliation where an increase in the frequency of leaf removal resulted in a reduced capacity for stored resources and associated growth rate of new leaves (Thornton & Millard, 1997; Anten *et al.*, 2003). More generally plastic responses of plants to environmental changes early in life may constrain responses to the same cue later on (Sultan, 2000; Weinig & Delph, 2001; Huber *et al.*, 2012). Probably the earlier responses entailed costs in terms of resource that limited later responses. It is further indicated that the extent to which early responses affect responses later in life is modulated by the internal nutrient status of the plant (Huber *et al.*, 2012).

In addition to the role of resource limitation suggested so far, it is also important to note that a relatively stronger elongation response in very small seedlings relative to later life stages might be adaptive, as such small plants are probably most negatively affected by burial. It is likely that in harsh drylands conditions the early period after germination is critical for survival (Li, 2010), and thus a strong phenotypic response in very young seedlings might have selected for.

Burial-induced stem elongation should be functionally coordinated with plasticity in other traits

Stem elongation in plants tends to be constrained by the requirements for

mechanical stability. Taller structures are subjected to larger bending moments and to avoid mechanical failure they must be designed with either broader bases or made of stronger and stiffer material. Thus, in order to meet these design requirements, shoot elongation should be accompanied by increases in stem diameter and/or stem tissue stiffness and strength to avoid mechanical damage (Niklas, 1992). Therefore a strong response of stem elongation may be expected to be functionally coordinated with plasticity in these other traits.

In the case of burial this issue is however somewhat complicated as burial itself reduces stem height above the soil (see also Fig. 1 in Chapter 5). But since plant stems are tapered the diameter of the basal stem section just above the soil surface will be smaller in a recently buried plant than in an unburied plant. So, if burial-induced stem elongation for example enables the plant to fully compensate its initial reduction in height, and if this is not associated with concomitant increase in stem strength, the plant will be mechanically more vulnerable. Thus, also in burial some degree of coordinated plasticity may be expected, at least from a mechanical perspective.

Consistent with this expectation I observed a stimulatory effect of burial on stem radial growth when comparing stem sections of the same developmental stage, indicating that plasticity in stem elongation was at least to some extent functionally coordinated with increases in diameter growth (Chapter 5). Contrary to my results for burial in Chapter 5, other studies found that shading induced the production of taller but thinner stems (Kennedy *et al.*, 2007; Anten *et al.*, 2009) and an associated higher ratio of height to diameter (Kennedy *et al.*, 2007). This lack of mechanically coordinated response in stem length and diameter growth under shading could be associated with the low resource availability in a shaded environment (typically shading experiments reduce light levels by more than 70%), which may result in mechanical instability (Anten *et al.*, 2009 and see further below).

In my study, the stimulatory effect of burial was insufficient to compensate for the above-mentioned tapering effect. However, as noted above in order to maintain mechanical stability increases stem elongation can also be associated with increases in tissue stiffness or strength. In Chapter 5, the Young's modulus (E), a measure of tissue stiffness, of *C. intermedia* was increased by burial in most of the seed families studied. In addition, final aboveground stem height was positively correlated with some other traits, e.g. diameter, total mass, breaking stress (σ_b), E , second moment of area (I) and flexural stiffness (EI). By contrast in *C. komarovii* (Chapter 2) burial decreased E , which was compensated by increase in diameter growth. Thus responses to burial in terms of stem length, diameter and tissue traits may differ among species.

Sand movement and the associated burial of plants strongly depends on factors such as vegetation cover, wind exposure, soil moisture and soil texture. These factors can all vary considerably on a relatively small spatial scale. However, the woody species used in my study commonly occur in different habitats varying from fixed to mobile sands. In these species intra-specific variation in responses to burial would thus be expected. Seeds of *C. intermedia* were collected from an area of about 3 km². Seed families of *C. intermedia* differed from each other in a number of traits, e.g. height, diameter, stem elongation height and diameter growth, total mass and a series of mechanical traits, when I compared them in a shade house experiment (i.e., under common conditions, Chapter 5). Especially notable was the almost two-fold variation in mean height growth among seed families. This suggests that there was a strong genetic variation in stem growth and mechanical traits among individuals even within a relatively small area (3 km²).

Interestingly, the effects of burial on stem mechanical traits (tissue stiffness, tissue strength and stem flexural rigidity) differed significantly among seed families as indicated both by the interaction of seed family and burial as well the difference in plasticity among seed families. The calculated stem elongation responses also seemed to vary considerably across seed families: ranging from no response to a more than 3-fold increase. But due to the limited replication in the study this difference was not statistically significant. Yet, my results clearly imply that there was indeed intra-specific variation in burial-induced responses in at least some of the stem traits. Intra-specific variation in plasticity in stem traits to other environmental factors such as shading (e.g. Dudley & Schmitt, 1995; Botto & Schmitt, 2002; Anten *et al.*, 2009) and flooding (Voesenek & Blom, 1998; Huber *et al.*, 2012) have been documented, but my study is the first that I know of to document such variation for burial.

It is interesting to note that the plasticity values for tissue traits correlated positively with the plasticity values for height growth. That is, the seed families that exhibited relatively strong increases in tissue strength and rigidity were also those that tended to have the largest responses in height growth. This suggests that variation in plasticity in different traits in response to burial is functionally coordinated and that responses may have co-evolved (Botto & Smith, 2002). Previous studies on intra-specific variation caused by shade avoidance in stem traits (stem elongation, Young's modulus and stem diameter; Anten *et al.*, 2009) and flowering acceleration (Botto & Smith, 2002) did not find such correlation between responses in different traits (Anten *et al.*, 2009). It is argued that a lack of such correlation indicates that microevolution in the

downstream pathways from signal perception has occurred separately (Botto & Smith, 2002). Possibly different microenvironment conditions, e.g. vegetation cover, wind direction and load affected by land configuration, soil texture and moisture, leads to genetic variation in phenotypic plasticity. According to ecological theory, it is considered that phenotypic plasticity is the main strategy of plants to adapt to the heterogeneous or continuously changing environment (Sultan, 1987; Alpert & Simms, 2002). Phenotypic plasticity of certain characteristics could also be passed to the next generation (Pigliucci, 2001), which possibly helps small seedlings survive in the harsh environment.

2. Mechanical stress

Plants in drylands ecosystems are commonly exposed to strong wind and in the presence of larger grazers to frequent trampling. Often plants cannot shield each other from wind and trampling. The mechanical impact of wind and trampling are very different. First, the force imposed by wind is usually small compared with the overwhelming force of trampling. Second, wind generally acts on the whole plant level but trampling is only acts at one point. Wind and trampling usually occur simultaneously with other stress factors, e.g. sand burial caused by strong wind or tissue browsing with trampling. The functional significance of plant responses to mechanical stress (thigmomorphogenesis) can thus not be viewed independently from other stress factors. In my study I thus investigated the interaction of different mechanical stresses (mild brushing mimicking wind effect and trampling) with other common stress factors in drylands dune ecosystem.

Effects mild mechanical stress (brushing), on growth

I used the method of brushing induced by a machine to simulate the mechanical effect of wind because real wind may change the microclimate around plants (temperature, evaporation and CO₂ concentration). This method also allowed me to flex many plants simultaneously at the same speed, time and force, while preventing the physical damage that often occurs when plants are manually flexed (e.g. Niklas, 1998; Anten *et al.*, 2005; 2009). Many previous studies found that mechanical stress negatively affected plant performance in terms of biomass production (Niklas, 1998; Wang *et al.*, 2008; Anten *et al.*, 2010), reproduction (Niklas, 1998; Cipollini, 1999) and/or stem elongation (Neel & Harris, 1971; Henry & Thomas, 2002). However, other studies (Anten *et al.* 2005; 2009; Liu *et al.*, 2007b) showed that mechanical

stress did not affect either growth rate or the final standing mass at the whole plant level. Some studies have even documented stimulatory effects of mild stress levels on growth, e.g. in: *Oryza saliva* (Michell, 1996), *Brassica oleracea* (Biddington & Dearman, 1985) and the tree species *Acer saccharinum* and *Liquidambar styraciflua* (Ashby *et al.*, 1979). It has been suggested that a moderate mechanical stimulus could promote assimilation and cell division, benefiting growth (Yang, 2002). In Chapter 2, brushing positively affected the growth of *C. komarovii* by increasing the net assimilation rate. Possibly the different effects of mechanical stress on growth depend on differences in plant type and intensity of stimulation applied.

Mechanical stress of brushing is strongly modified by sand burial

As noted, in areas with moving sands, wind and sand burial tend to occur simultaneously and it is therefore important to consider whether the effect of one depends on the level of the other. As burial raises the soil level, the part of the stem that is above the soil will inevitably be shorter, thinner and younger in a buried than an unburied plant. As burial covers some of the leaves it also reduces wind drag area. Together this entails that at a given wind speed buried plants experience smaller bending moments than unburied ones. However, as noted above due to stem tapering, partially buried plants also have smaller basal stem diameters while they respond to burial through increased elongation. Finally, the partial burial itself may affect curvature in bending when plants are exposed to a lateral force such as wind (Gere & Timoshenko, 1999). Thus, the stress distribution along the stem induced by wind drag can differ considerably between partially buried and unburied plants. It may therefore be expected that the effects of mechanical stress on stem and whole-plant mechanical traits differ with the presence or absence of sand burial. In Chapter 2, it was found that in partially buried *C. komarovii* plants brushing induced a considerable increase in stem diameter growth and tissue stiffness (E), leading to a more than two-fold larger rigidity of basal cross section (flexural stiffness; EI) and a larger force to resist stem rupture. For unburied plants, these effects on E , EI or the lateral force in response to mechanical stress were not observed. Thus, the effect of brushing indeed seemed to strongly depend on the level of burial.

These differential responses could in the first place be associated with the above-mentioned differences in stress distribution caused by brushing in buried and unburied plants. The brushing treatment entailed bending stems to a certain angle which requires a larger force in shorter stems than in longer ones, and this may produce a larger stress. Alternatively the different

responses to brushing may be associated with the fact that, compared with unburied plants, the basal section of the aboveground shoot of a buried plant is relatively younger. It has been shown that younger tissues tend to be more plastic in their responses to external stimuli including mechanical stress than older tissue (Biddington, 1986; Chehab *et al.*, 2009). In the drylands dune ecosystem, plants are exposed to frequent wind loading varying both in time and space. Possibly low levels of mechanical stress serve as a cue to adjust allometric growth patterns in order to avoid future mechanical damage when stronger forces may occur, especially for partly buried plants. As discussed above, stem elongation under burial was associated with plastic shifts in other traits. The responses to environmental factors may be modulated by the conditions induced to plants at early developmental stages (Chapter 5). Strong interactive effects of burial and brushing occurred in both morphological and mechanical traits (Chapter 2). These plastic responses are expected to be coordinated. Besides, mechanical stimulation may give relatively stronger responses in buried seedlings relative to later life stages, and this might be adaptive.

Effects of water availability and its interaction with trampling

Water is an important factor limiting plant performance in arid and semi-arid regions (Hanke *et al.*, 2011). With sufficient water availability plants may allocate more mass to stems, affecting height, diameter and tissue rigidity, and this has consequences for the plant's resistance to mechanical stress. Furthermore, shifts in allocation to the root system may affect the anchorage strength of plants (Goodman & Ennos, 1996). In drylands resources are strongly heterogeneously distributed (Housman *et al.*, 2007). The heterogeneity possibly leads to a variation in phenotypic plasticity of species with different drought tolerance. Relatively wet patches may attract more livestock as a result of a high vegetation cover and the possible presence of drinking spots. Such circumstances may entail that soil water availability and probability of trampling are positively correlated.

High water availability may result in taller and more massive plants which suggests that the impact of mechanical stress on such plants would be larger, though this could partly be mitigated by the fact that plants at high water availability also make thicker and stronger stems.

Consistent with previous researches on herbaceous species (Sun & Liddle, 1993a; Andrés-Abellán *et al.*, 2006; Hill & Pickering, 2009), in my study trampling decreased stem length and vertical height but increased radial stem growth of the four shrub species at two water levels. Contrary to my

expectation, for all species the effect of trampling was similar at different water availabilities. My results are consistent with another study (Wang *et al.*, 2008) on the effects of another form of mechanical perturbation (shaking) on the stem rigidity of *H. laeve* (Wang *et al.*, 2008). However, in the herb *Corispermum mongolicum* the direction of plant responses to mechanical stress (shaking) differ in direction depending on water availability. At low water availability, mechanical stress induced reductions in stem rigidity while the opposite patterns was found at high water availability (Wang *et al.*, 2009b). This discrepancy may reflect structural differences among woody shrubs (Wang *et al.*, 2008 and this thesis) and herbaceous plants (Wang *et al.*, 2009b). Turgor pressure plays a much more important role in resistance to mechanical stress by determining stem stiffness in herbaceous plants than in woody species (Struik, 1965). Probably the composition and quantity of tissue (e.g. parenchyma, sclerenchyma, xylem, vascular tissue, and so on) is more crucial for woody plants than turgor pressure. Thus, it is possible that in herbaceous plants mechanical stress-induced changes in tissue stiffness are mediated by changes in turgor pressure which in turn is directly related to water availability.

Effects of trampling in clonal and non-clonal plants

Trampling is a common phenomenon in drylands ecosystems (Hanley & Sykes, 2009; Sørensen *et al.*, 2009; Gruntman & Novoplansky, 2011). It usually occurs during grazing and simultaneously with defoliation. In Chapter 4, I analyzed the effects of repeated trampling (three times a week, for 14 weeks) in four typical species from the Mu Us Sandland: *Caragana intermedia*, *Cynanchum komarovii*, *Hedysarum laeve* and *Hippophae rhamnoides*. I used a device that simulated the impact of trampling by sheep: one of them most common grazers in the Mu Us Sandland. This treatment had no effect on the survival of the seedlings of any of the four species, and only decreased biomass of *H. rhamnoides* at both high and low water availability, suggesting that seedlings of these woody species, except of *H. rhamnoides*, were tolerant to trampling.

In Chapter 3, I investigated whether trampling tolerance is associated with clonal integration in plants. As noted in Chapter 1 many of the woody species in the Mu Us Sandland are clonal: i.e., they have the capacity to produce vegetative offspring (ramets) along horizontal runners. To address this question I conducted a field study with *Populus simonii* a clonal root-suckering tree. New ramets are produced on horizontally extended roots. In drylands dune ecosystems, population growth of clonal species is mainly

determined by successful establishment of vegetative offspring (Li, 2010). Compared to seedlings coming from seeds, clonal ramets can exchange resources through clonal connections, and this may enable them to resist external stress and may thus increase survival and growth (Guàrdia *et al.*, 2000; Yu *et al.*, 2004, 2008; Li, 2010). Trampling had strong negative effects on survival but this effect was much larger in experimentally disconnected than in connected ramets. These results showed that clonal integration can be important in determining trampling tolerance. I also applied a defoliation treatment in this experiment and interestingly its effects were independent of whether plants were disconnected. As noted, trampling is one of the two main stress effects associated with grazing the other being tissue loss. Thus, my results suggest that in *Populus simonii* the positive effect of clonal integration on grazing tolerance results from it mitigating the impact of grazing-associated trampling and not through grazing-associated tissue loss. Previous studies have documented that clonal integration may increase tolerance to several stress factors including shading (Alpert, 1999; Alpert *et al.*, 2003), water shortages (de Kroon *et al.*, 1996; Dong & Alaten, 1999), nutrient depletion (Alpert, 1991), burial (Yu *et al.*, 2004), erosion (Yu *et al.*, 2008). The current study is the first to document that it may also enhance trampling tolerance and in this way indirectly grazing tolerance .

So why did clonal integration play an important role in mitigating trampling effects? Mean precipitation in the research region where I conducted my study is low (260 – 450 mm annually) and many plants grew in almost pure sand. As a result they probably experienced water limitations. For *P. simonii*, the amount of fibrous roots is important in determining survival of disconnected ramets. However, trampling possibly directly damages the existing root system and leads to reduction in root growth, which could be more severe in woody species than in herbaceous ones as a result of relatively rigid stems of woody plants. Trampling may induce a large bending moment on the root system in turn causing root damage. Fibrous roots in sandy soils tend to have low shear strengths (Berry *et al.*, 2004). Together these factors contribute to the low root anchorage strength (Crook & Ennos, 1994; van Delden *et al.*, 2010). Thus we suggest that trampling exacerbated drought stress by causing root damage, and this effect can also occur in other non-clonal woody species.

Trampling induced flexibility vs. strength trade-off

In Chapters 3 and 4, I quantified how plastic responses in stem traits affected mechanical behavior at the whole-shoot level. There are basically two ways by which plants can reduce the chance of damage under external forces: they can

increase their resistance to breakage or enhance their flexibility to minimize the forces encountered (Puijalon *et al.*, 2008; Paul-Victor & Rowe, 2011; Puijalon *et al.*, 2011). I quantified the former strategy as the minimum external force (F_{break}) required to rupture the stem at its base (Anten *et al.* 2005) and the latter strategy as the inverse of the force needed to bend the stem ($1/F_{\text{bend}}$). The ratio $F_{\text{break}} / F_{\text{bend}}$ can thus be considered a rough estimate of the balance between strength and flexibility. As noted earlier contrary to wind loading, trampling of small plants by large grazers entails an overwhelmingly large force, implying that flexibility rather than strength should be expected to increase trampling tolerance (Sun & Liddle, 1993a).

Contrary to this expectation the general results of Chapters 3 and 4 were that trampling increased both F_{break} (resistance to rupture) and F_{bend} (resistance to bending). But the effect on F_{bend} was stronger than the effect on F_{break} resulting in lower $F_{\text{break}} / F_{\text{bend}}$ ratios, reflecting a less favorable balance between strength and flexibility. Increase in strength at the expense of flexibility in response to trampling seems maladaptive as trampling represents an overwhelming force. This response to trampling however is similar to the observed responses of plants to milder forms of mechanical stress (e.g., wind, rubbing and flexing; Biro *et al.*, 1980; Telewski, 1990; Anten *et al.*, 2005) in which case increased strength is a feasible strategy to avoid mechanical damage. It is therefore possible that in the species observed by me different forms of mechanical stress induce qualitatively similar responses, and that these responses improve tolerance to wind loading but not trampling.

Finally as noted above, in addition to reductions in $F_{\text{break}} / F_{\text{bend}}$ plants also responded to trampling through increased stem declination a response that helps reduce the chances of mechanical damage under trampling as the bending required to press the plant shoot to the soil becomes smaller (Sun & Liddle, 1993a).

3. Defoliation

Overgrazing is considered to be the major cause of desertification and grassland degradation worldwide (Squires, 2003; Yoav *et al.*, 2006; Verón & Paruelo, 2010). In addition to trampling, loss of leaf area (defoliation) is one of the main effects of grazing (McNaughton, 1983). Defoliation entails a reduction in photosynthetic capacity and loss of resources and may thus negatively impact plant performance. Compensatory growth after defoliation however enables many plant species to mitigate these negative effects (McNaughton, 1983; Anten *et al.*, 2003; Gruntman & Novoplansky, 2011).

This compensatory growth is thus the primary mechanism determining defoliation tolerance.

There is currently a strong debate about the extent to which compensatory growth after defoliation is affected by other factors. While limitations of soil resources tends to positively affect compensatory growth (Coughenour *et al.*, 1990; van Staalduinen & Anten, 2005), shading can strongly aggravate the negative effects of defoliation (Anten *et al.*, 2003). In this thesis I primarily focused on the interactive effects between defoliation and sand burial.

For *C. komarovii*, defoliation itself had a small negative effect on growth. This negative effect was strongly aggravated by burial (Chapter 2). In a separate experiment in collaboration with Ineke Roeling (Utrecht University), I subjected a woody species, *Artemisia ordosica*, to different levels, 0, 25%, 50%, 75% and 100% of defoliation (Fig. 2 in current Chapter). When applied alone both defoliation and burial stimulated relative growth rates, both expressed in terms of mass (RGR_m). However, when defoliation was applied in combination with burial it had a negative effect on RGR_m (Fig. 2 in current Chapter). Again, this indicates that the capacity for compensatory growth after defoliation, as well as the associated defoliation tolerance, is reduced by burial. This result can be explained by the limiting resource model (LRM; Wise & Abrahamson, 2007) indicating that tolerance to herbivory depends on the type of resource and herbivore. Defoliation directly affects light acquisition, which should be expected to be aggravated by burial. In mobile or semi-fixed dunes, sand movement and the resulting burial of plants are primarily due to overgrazing-induced desertification. My results suggest that burial and grazing-induced defoliation can aggravate each other's effect, and this should be taken into account when considering the sustainability of grazing and the potential for vegetation collapse in dry regions (see section 4 Contributions).

Table 1. Results of one-way ANOVA for *Artemisia ordosica* in only burial or defoliation, and results of two-way ANOVA for the combination of burial and defoliation.

	RGR _h	RGR _m	LAR	NAR
One-way ANOVA				
Burial	571.17 ^{***}	9.61 ^{***}	5.79 ^{**}	9.09 ^{***}
Defoliation	0.68 ^{ns}	5.62 ^{**}	19.15 ^{***}	57.30 ^{***}
Two-way ANOVA				
Burial	126.62 ^{***}	0.05 ^{ns}	6.34 [*]	9.54 ^{**}
Defoliation	3.74 ^{ns}	1.57 ^{ns}	27.66 ^{***}	55.90 ^{***}
Bu×De	2.22 ^{ns}	15.02 ^{***}	0.67 ^{ns}	0.11 ^{ns}

F values and significance levels (^{***} $P < 0.001$, ^{**} $P < 0.01$, ^{*} $P < 0.05$ and ^{ns} $P \geq 0.05$) are given.

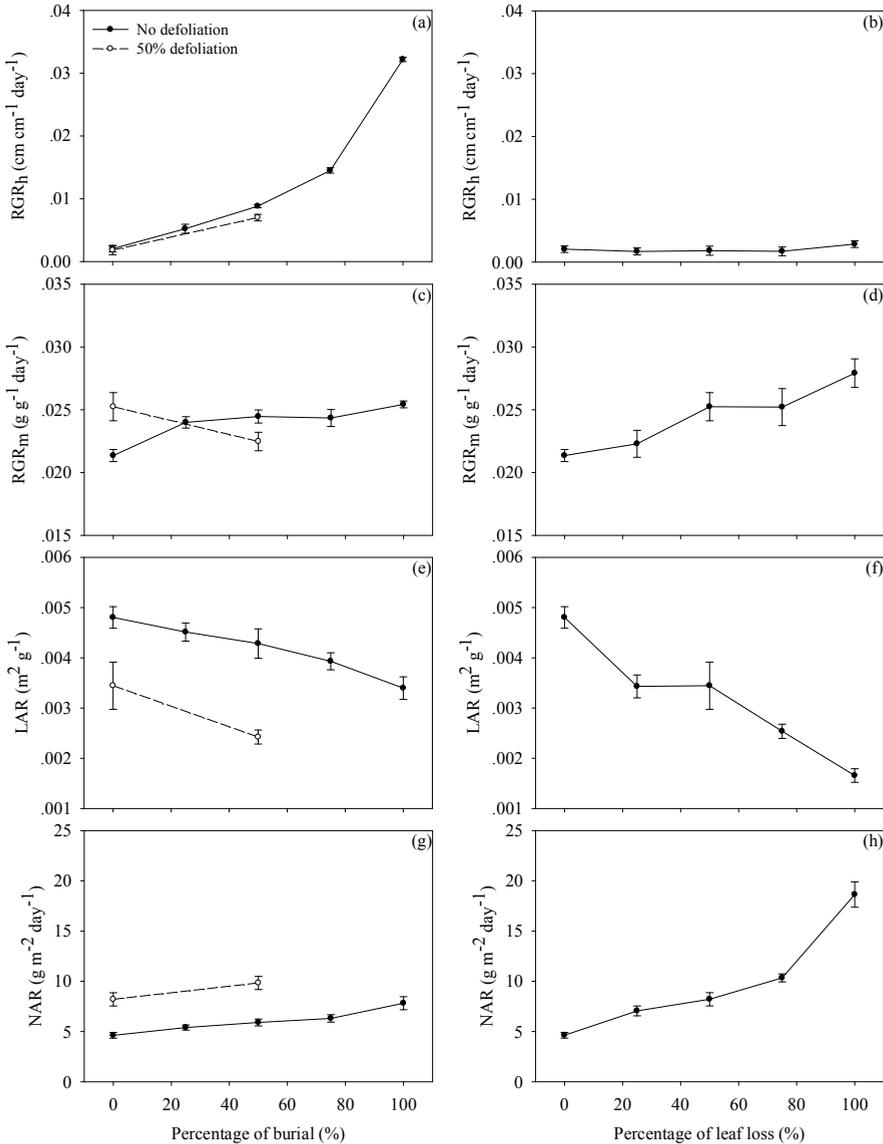


Fig. 2. Different degrees of burial and defoliation treatments were applied to *Artemisia ordosica* plants. For burial, 0, 25%, 50%, 75% and 100% of the aboveground shoots were covered by sand. An interactive treatment was applied by firstly removing 50% of the leaves evenly and then burying the shoot to 50% of its height. For defoliation, 0, 25%, 50%, 75% and 100% of leaves were removed evenly. Relative growth rates of height, relative growth rates of mass, leaf area ratios and net assimilate rates of burial and associated defoliation-burial (a, c, e and g) and defoliation and associated burial-defoliation (b, d, f and h) are showed. Data are means \pm SE. Data obtained in collaboration with Ineke Roeling.

4. Contributions to management in desertification control

Worldwide grassland degradation and desertification are major problems in drylands, affecting hundreds of millions of people (Squires, 2003; Yoav *et al.*, 2006; Verón & Paruelo, 2010). The region where this research focused on, Inner Mongolia, has suffered severe desertification for several decades (Zhang, 1994, van Staaldin & Werger, 2006). Desertification probably involves a number of factors acting in concert. Overgrazing, through defoliation and trampling, reduces plant growth and thus the vegetation cover. Wind in areas with an exposed sand surface results in sand movement and leads to burial or denudation. This may occur in a fine-grained pattern. In this thesis I studied the effects of these factors (except denudation) on plant traits and performance, with special emphasis on interactions between stress factors. Here, I consider the utility of my results for an appropriate management of desertification control.

Clonal plants are widely used for stabilization of mobile and semi-fixed dunes and restoration of vegetation (Zhang *et al.*, 2002; Yu *et al.*, 2004; Li *et al.*, 2008), which together form a key element in combating desertification. As noted, clonal plants form vegetative offspring (ramets) along horizontal runners and thus have the ability of sharing resources and local specialisation of ramets in a connected network. Physiological integration can enhance the performance of ramets suffering from different stresses, including shading (Alpert, 1999; Alpert *et al.*, 2003), water shortages (de Kroon *et al.*, 1996; Dong & Alaten, 1999), nutrient depletion (Alpert, 1991), burial (Yu *et al.*, 2004), erosion (Yu *et al.*, 2008). A primary finding in this thesis is that clonal integration may be an important trait associated with grazing tolerance, but that this is primarily due to this integration mitigating the negative effects of grazing-associated trampling rather than grazing-associated tissue loss. Trampling without tissue browsing could also bring great harm to the plants., Damage caused by trampling strongly declines with increasing plant size. Especially, plants beyond a certain height are unlikely to be trampled. That means that in clonal plants larger ramets suffer little or no trampling and they can mitigate the damage imposed by trampling on their small vegetative offspring. More generally, in the developing sustainable grazing methods, control of trampling might be at least as important as reduction of tissue consumption.

In the dunes, the growth pattern of plants is probably related to the mobility of sand along the dune fixation process. For example, *Artemisia ordosica* occurs in different habitats but tends to show lower growth rates in fixed than

in more mobile dunes (Li, 2010). Absence of burial may decline the vigor in some dune species (Dech & Maun, 2006; Li, 2010). Thus it appears that some non-clonal species such as *A. ordosica* grow particularly well by partial burial in (semi-) mobile dunes. Such species should be used as a first line approach to fix dunes.

Though the existence of burial may stimulate the growth, the interaction with other stress factors may result in adverse effects. Defoliation combined with subsequent burial had negative effects on plant growth. Less photosynthetic area and resource remobilization of buried leaves together resulted in that negative growth effect. Trampling occurring simultaneously with defoliation is likely to aggravate the damage. These results point to the need of strongly restricting or even prohibiting grazing of vegetation on mobile or semi-fixed dunes where sand movement is frequent.

In dune ecosystems, some non-clonal species have been shown to be tolerant to environmental stress (Li, 2010). Though young seedlings may show low growth and survival rates at their early stages, those that do pass this stage have high survival probability at later life stages (Li, 2010). In Chapter 5, it is shown that plastic responses to sand burial vary within species. It is likely that the individuals with rapid shoot elongation and strong resistance to mechanical rupture will perform better under burial. More work is needed to investigate the extent and ecological meaning of this variation in plastic responses to burial and other stress factors. However this variation could have important implications for management. For example, plants with greater tolerance to burial could be selected for seed culture and then used for future dune fixation and vegetation recovery.



Sand movement in mobile dune.

Samenvatting

Deze dissertatie gaat over de reactie van dominante houtige planten in het zandgebied van Mu Us, Binnen-Mongolië, China, op een aantal plaatselijk beperkende milieufactoren, waaronder droogte, begrazing, overstuiving, mechanische belasting als gevolg van wind of betreding, en de interactieve effecten van deze factoren. Ook wordt de betekenis van fysiologische integratie in clonale planten voor het verzachten van de negatieve gevolgen van deze beperkende milieufactoren onderzocht. Naast de fenotypische plasticiteit van de soorten wordt ook de intraspecifieke variatie in plasticiteit geanalyseerd, evenals de mate waarin verschillende plantkenmerken reageren en de mate waarin ze functioneel gecoördineerd zijn. Tenslotte worden de resultaten besproken in de context van management en vegetatieherstel.

Overstuiving

In droge gebieden leidt overbeweiding, en ook klimaatsverandering, tot de vorming van kale plekken in de vegetatie en bij sterke wind kan dit tot zandverstuiving leiden, waardoor planten overstoven kunnen worden. Als planten diep en/of langdurig begraven raken kan dat fataal worden. Toenemende bovengrondse hoogtegroeï, waarbij een plant het aantal bovengrondse bladeren weer op peil brengt, is een belangrijke reactie op overstuiving. Hieraan wordt in deze dissertatie veel aandacht besteed.

Cynanchum komarovii, *Caragana intermedia* en *Artemisia ordosica* verschilden in de mate waarin de hoogtegroeï van de planten toenam na overstuiving. Verder hangt de mate van toenemende hoogtegroeï af van het ontwikkelingsstadium van de plant en van de mate van vorige overstuivingen: een eerste overstuiving van 2 maanden oude planten van *C. intermedia* leidde tot een veel sterkere hoogtegroeï dan een tweede overstuiving een maand later. Mogelijk is dit een gevolg van beperkte voorraden in de plant. Stengelverlenging als gevolg van overstuiving leidt hoogstwaarschijnlijk tot de reallocatie van voorraden uit de begraven bladeren, en na eerdere overstuiving kunnen die voorraden uitgeput raken. Een dergelijk patroon is ook voor andere plastische reacties te verwachten.

Stengelverlenging hoort samen te gaan met een toename in de stengeldiameter en/of in de stijfheid en sterkte van stengelweefsel teneinde mechanische schade te beperken. Radiale stengelgroei nam in *C. intermedia* na overstuiving toe, maar dat was niet genoeg om de stengeldiameter aan het nieuwe bodemoppervlak

even groot te maken als de stengeldiameter aan het oude bodemoppervlak vóór de overstuiving. De plant is daardoor dus mechanisch kwetsbaarder geworden. De Modulus van Young (E), een maat voor weefselstijfheid, nam na begraving in bijna alle zaadfamilies van *C. intermedia* toe en bovengrondse stengellengte bleek positief gecorreleerd met een aantal plantkenmerken die verband houden met de stevigheid van de plant. In begraven *C. komarovii* daarentegen nam E af maar dit werd gecompenseerd door de toename in diametergroei van de stengel. De reacties op overstuiving in termen van stengellengte, diametergroei en weefselkenmerken kunnen dus van soort tot soort verschillen. In de zaadfamilies van *C. intermedia* bleken de kenmerken van stevigheid en de mate van hoogtegroei gecorreleerd, hetgeen suggereert dat de variatie in de plasticiteit van de diverse kenmerken functioneel gecoördineerd is.

Mechanische belasting

In droge gebieden staan planten vaak bloot aan sterke wind en aan betreding door grote grazers. De mechanische effecten van windbelasting en betreding zijn sterk verschillend. Allereerst is de kracht die de wind op de plant uitoefent gewoonlijk veel geringer dan de kracht die betreding uitoefent. Bovendien werkt wind op de hele plant en betreding gewoonlijk op een deel van de plant. Windbelasting gebeurt vaak in combinatie met overstuiving, betreding vaak in combinatie met het afbijten van plantenmateriaal. Daarom is het vaak weinig zinvol om de effecten van mechanische belasting op de plant (thigmomorphogenese) onafhankelijk van andere beperkende factoren te evalueren.

Windbelasting werd nagebootst door planten machinaal te borstelen, omdat echte wind ook het microklimaat rond de plant ingrijpend zou veranderen. Borstelen had een positief effect op de groei van *C. komarovii* doordat de netto assimilatiesnelheid toenam.

In zandverstuivingen gebeuren windbelasting en overstuiving vaak tegelijkertijd en het is interessant de effecten ervan goed te analyseren. Na overstuiving is het bovengrondse deel van de plant onvermijdelijk korter, dunner en jonger dan voor de overstuiving. Bovendien zijn er bladeren begraven en dat reduceert de windbelasting op de plant. Als gevolg van beide effecten ondervinden gedeeltelijk overstoven planten geringere buigmomenten dan niet-overstoven planten. De belasting langs de stengel als gevolg van de kracht van de wind verschilt vaak sterk tussen een gedeeltelijk overstoven en een niet-overstoven plant. Het borstelen van gedeeltelijk overstoven *C. komarovii* planten induceerde een aanzienlijke toename in stengeldiameter en

weefselstijfheid E , resulterend in een meer dan twee keer zo grote rigiditeit EI van de stengelbasis en dus een grotere sterkte om breuk te weerstaan. Niet-overstoven planten lieten niet zo'n reactie op mechanische belasting zien. Het effect van borstelen lijkt dus sterk afhankelijk te zijn van overstuiving.

Water is een groeibeperkende factor in droge gebieden en dat heeft weer gevolgen voor de mechanische stabiliteit van de plant. In planten van *Caragana intermedia*, *Cynanchum komarovii*, *Hedysarum laeve* en *Hippophae rhamnoides*, die onder twee niveaus van waterbeschikbaarheid groeiden, leidde betreding tot een kortere stengellengte en een grotere stengeldikte, onafhankelijk van het niveau van waterbeschikbaarheid. Uit de literatuur blijkt dat dit voor kruiden anders kan zijn. In de dissertatie worden de aspecten hiervan besproken.

In Mu Us groeien veel houtige planten clonaal. Een geïntegreerd clonaal systeem biedt de mogelijkheid tussen de ramets stoffen uit te wisselen en dat kan van voordeel zijn voor klonen die in een heterogeen milieu groeien. Betreding bleek een sterk negatief effect op de overleving van ramets van *Populus simonii* te hebben, en dit effect was veel minder sterk in clonaal verbonden ramets dan in ramets waarvan de clonale verbindingen doorgesneden waren. Het effect van experimentele ontbladering van die ramets bleek echter niet te verschillen tussen verbonden en gesepareerde ramets. Het lijkt er dus op dat bij *Populus simonii* clonale integratie de tolerantie tegen begrazing verhoogt omdat de effecten van betreding gemitigeerd worden en niet zozeer die van ontbladering.

Plastische reacties in stengelkenmerken hebben invloed op de mechanische stevigheid van de plant als geheel. In principe kunnen planten op twee manieren de kans op schade als gevolg van mechanische belasting verkleinen: ze kunnen hun breekweerstand verhogen of hun flexibiliteit. De mechanische sterkte wordt gekarakteriseerd door de minimale externe kracht (F_{break}) die de stengel doet breken en de inverse van de kracht ($1/F_{bend}$) die nodig is om de stengel een beetje te buigen. De verhouding F_{break}/F_{bend} is dan een ruwe schatting van de balans tussen sterkte en flexibiliteit. Betreding door grote grazers betekent een dusdanig sterke kracht op de plant dat flexibiliteit, en niet sterkte, hier een belangrijke eigenschap lijkt. Betreding bleek echter zowel F_{break} als F_{bend} te doen toenemen, maar vooral F_{bend} , zodat F_{break}/F_{bend} kleiner werd. Dit resultaat wordt in de dissertatie vergeleken met in de literatuur gemelde resultaten.

Ontbladering

Overbeweiding is een belangrijke oorzaak van desertificatie. Betreding en ontbladering zijn de belangrijkste effecten van beweiding. Ontbladering impliceert een afname van de fotosynthetische capaciteit en een verlies van biomassa en nutriënten en kan de plantengroei dus negatief beïnvloeden. Maar

compensatoire groei na begrazing stelt de plant in staat dergelijke negatieve effecten te reduceren.

In *C. komarovii* had ontbladering slechts een klein negatief effect op de groei, maar het effect werd veel sterker in combinatie met overstuiving. *Artemisia ordosica* planten werden voor respectievelijk 0, 25, 50, 75 en 100 % ontbladerd. Ontbladering als zodanig deed de relatieve groeisnelheid toenemen, net als overstuiving dat deed. Maar als ontbladering en overstuiving gecombineerd werden nam de relatieve groeisnelheid af. Overstuiving lijkt dus de capaciteit voor compensatoire groei na ontbladering te verminderen. Dit resultaat is in het licht van het 'Limiting Resource Model' te begrijpen. In combinatie hebben overstuiving en ontbladering (begrazing) dus een sterk negatief effect op de plantengroei.

Beheer

Clonale planten worden veel gebruikt om mobiele en half-gefixeerde duinen te stabiliseren en vegetatie te herstellen. Een belangrijk resultaat gerapporteerd in deze dissertatie is, dat clonale integratie belangrijk kan zijn voor begrazingstolerantie in planten, en dan eerder omdat clonale integratie de effecten van betreding mitigeert dan die van weefselverlies. Maar alleen al de effecten van betreding kunnen een sterk negatief effect op planten hebben. Bij het ontwikkelen van duurzame beweidingmethoden moet er dus voldoende aandacht voor zowel betreding als weefselverlies zijn.

Planten reageren erg verschillend op de mobiliteit van hun substraat. Sommige duinsoorten nemen in groeikracht af als ze niet meer regelmatig overstoven worden. *Artemisia ordosica*, bijvoorbeeld, groeit vooral goed onder gedeeltelijke overstuiving in een mobiel tot half-gefixeerd duinlandschap. Zulke soorten kunnen dus goed gebruikt worden om mobiele duinen enigszins te stabiliseren. Maar, zoals beschreven in deze dissertatie, dan moet het gebied niet of nauwelijks beweid worden, want combinatie van ontbladering en overstuiving, of van betreding en ontbladering, verslechtert de groei van dergelijke planten sterk.

De plastische reacties op overstuiving, ontbladering en betreding van de verschillende soorten verdienen meer onderzoek, omdat begrip daarvan erg nuttig kan zijn bij het kiezen van soorten of zaadfamilies die ingezet gaan worden bij het fixeren van mobiele duinen en het herstel van een begroeiing.

摘要

本论文研究了内蒙古地区毛乌素沙地主要木本植物对干旱区沙地生态系统的常见环境胁迫因子, 干旱, 放牧, 沙埋, 机械刺激 (包括风力和践踏)等及其交互作用的生态适应机制; 阐述了克隆整合在克隆植物抵抗胁迫因子影响过程中的作用; 不仅比较了不同物种的种间表型可塑性, 还分析了同一物种的种内可塑性变化; 探讨了植物不同特征变化间的功能协调性; 最后讨论了研究结果在实际植被恢复过程中应用。研究框架和主要结论详见图 1。

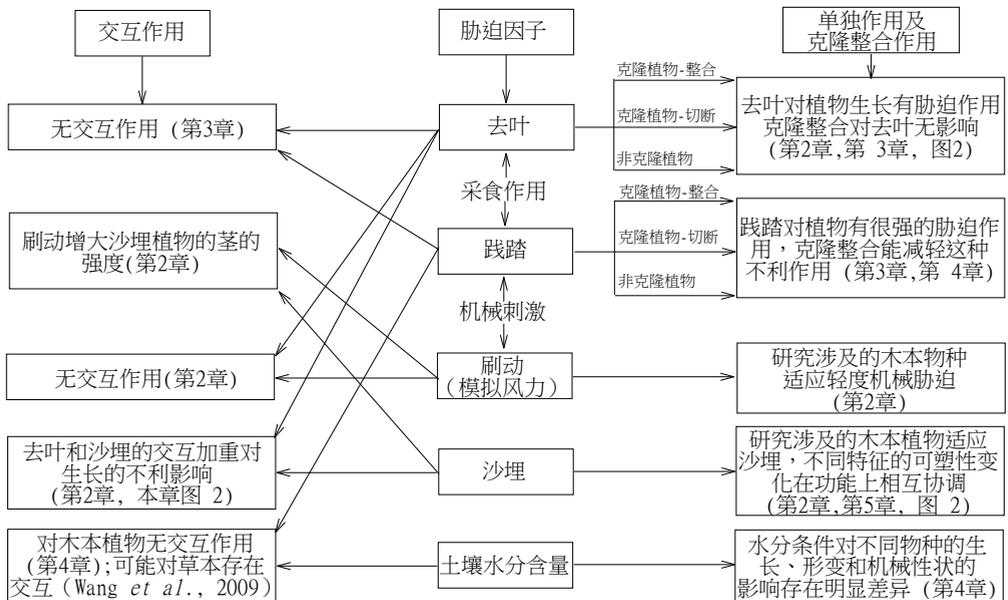


图 1. 本研究涉及的干旱区生态系统主要环境胁迫因子及其交互作用对木本植物的影响。

1. 沙埋

在干旱地区, 过度放牧和/或气候变化引起严重的植被退化, 失去保护的沙地表面直接暴露于风力的侵蚀, 从而导致表面沙层的移动并掩埋残存植被。沙埋直接减少植物的有效光合面积和根系供氧量, 并阻碍植物茎垂直方向的生长。沙埋超过一定的地上茎高度或一定时间会导致植株的死亡 (Maun, 2004)。不同物种对沙埋的耐受能力有显著差异 (Brown, 1997; Maun, 1998; Dech & Maun, 2006; Liu et al., 2008)。地上茎在垂直方向的快速生长是植物对沙埋的主要反应之一, 增加的地上高度为新叶片的生长提供了空间 (Voesenek et al., 1998; Dech & Maun, 2006; Gilbert & Ripley,

2008; Jia *et al.*, 2008; Liu *et al.*, 2008)。此外, 植物对沙埋的耐受能力也受到其他一些生长特性的影响, 如不定根的生长, 叶片光合能力的增强, 以及被沙埋与未被沙埋部分间的资源再分配(Dech & Maun, 2006; Perumal & Maun, 2006; Gilbert & Ripley, 2008)。本研究主要关注的是沙埋引起的地上茎的生长。

不同生长阶段对地上茎生长的影响

植物茎在垂直方向的快速生长是植物应对沙埋的重要和普遍反应之一, 本研究涉及的木本植物在遭受沙埋时也有相同表现(牛心朴子, 第2章; 柠条, 第5章; 油蒿, 本章图2), 此外在油蒿的沙埋试验中还发现其地上茎生长速度随着沙埋水平的增大而加快(本章图2)。

沙埋引起的茎快速生长的生理机制仍有待进一步研究, 乙烯浓度的累积被认为是其中一个重要因素(Voesenek & Blom, 1998; Voesenek *et al.*, 1998)。乙烯对茎生长的促进作用在其他研究中已被证实(Pierik *et al.*, 2004)。沙埋对植物的机械刺激作用促进了乙烯的生成, 而沙埋本身在一定程度上抑制了乙烯的挥发, 从而有利于乙烯量的累积。另一方面, 有研究显示乙烯促进茎的垂直方向生长是通过增加茎节间数而非增加节间长度(Voesenek *et al.*, 1998)。其他一些因素, 如遮阴(沙埋造成植物某些部分处于完全黑暗环境)和二氧化碳浓度的累积, 也被认为对茎的生长具有一定影响(Voesenek *et al.*, 1998)。

本研究还发现沙埋对茎生长的促进作用取决于植物所处的生长阶段和/或之前遭受沙埋的经历。第5章中, 两个月大小的柠条幼苗在被施加第一次沙埋处理后, 其茎生长速度比未沙埋的植株平均快了约53%; 而一个月之后的第二次沙埋只产生了约10%的差异。沙埋引起的茎的生长涉及到各器官间的养分转移和再分配(Freas & Kemp, 1983; Gilbert & Ripley, 2008), 这一功能可能会随着沙埋次数的增加而减弱。在对胁迫因子去叶的重复性作用研究中也发现植物自身养分储存和新叶片生长能力随着去叶频率的增大而减弱(Thornton & Millard, 1997; Anten *et al.* 2003)。一般来说, 因植物对早期环境胁迫因子的塑性反应产生的资源消耗会限制之后对同一因子的类似反应(Sultan, 2000; Weinig & Delph, 2001; Huber *et al.*, 2012)。这种对同一胁迫因子的不同强度的反应可以通过控制前后刺激期间的养分水平加以调节(Huber *et al.*, 2012)。

茎的生长与其他特征的功能协调性

从机械性状角度出发, 植物茎的高度生长受限于其稳定性。即地上部分越高, 其所需承受的弯曲力矩越大, 从而需要更大的茎宽或/和更大的机械强度来支持地上部分的重量。因此, 茎的生长同时伴随着茎宽和/或者茎强度的增大以避免可能的机械损伤(Niklas, 1992)。

沙埋直接减小了植物地上部分高度以及基径(图1, 第5章)。茎的快速生长部分补偿了沙埋带来的地上高度的减小。如果其他性状没有产生相应的变化以支持快速增高的地上部分, 那么地上茎可能会因为发生弯折而受到机械损伤。因此, 沙埋植株的一些特征变化在功能上应是相互关联的。

第5章中,被沙埋植株的基径生长速度小于对照植株,但大于对照个体的初始高度三分之二处,即与沙埋基径同一相对位置处的生长速度。在遮阴处理对植物生长的研究中发现与对照相比,被遮阴处理的植株地上茎相对较高但基径相对较小(Kennedy *et al.*, 2007; Anten *et al.*, 2009),因此导致了较大的高茎比(Kennedy *et al.*, 2007)和机械性状上的不稳定性(Anten *et al.*, 2009)。第5章中沙埋对基径生长的促进作用不足以补偿高度快速生长带来的构型上的不平衡,沙埋刺激了被沙埋植株的地上茎强度的增大(用杨氏模量表示)以弥补构型上的不足。植物地上高度变化和其他一些特征变化相互关联,如基径,总生物量,断裂压力,杨氏模量,面积二次矩和弯曲刚度等。第2章中,被沙埋的牛心朴子的杨氏模量小于对照,但较快的基径生长补偿了茎强度的减小。因此与沙埋引起的茎高度生长、基径生长和强度变化情况因物种而异。

沙层在风力作用下的移动速度取决于很多因素,如植被覆盖度,风速,土壤类型和湿度等。沙地生态系统中,这些因素在时空分布上存在很大差异。本研究中涉及的木本植物广泛分布于各种不同类型的沙地。生存环境间的差异导致了对同种胁迫因子的种内差异响应。第5章中的柠条种子从约3平方公里范围的沙地收集而来。源自不同母株的植物(不同种组)在很多特征上存在差异,如生物量,机械性状,高度和基径及其生长速度等。以平均高度生长速度为例,不同种组间最大相差约两倍。这表明在一个相对较小的沙地生态系统范围内,同一物种不同个体的茎生长和机械性状存在明显的种内遗传变异。沙埋和种组的交互总用以及种组间的塑性变化(第5章)说明沙埋对茎的机械性状有显著影响。

本研究还发现高度生长的可塑性变化与茎的机械性状可塑性变化呈正相关关系,生长最快的种组其茎的强度也最大,说明沙埋引起的不同生长特征的变化在功能上相互协调(Botto & Smith, 2002)。根据相关生态学理论,表型可塑性是植物的适应环境变化的主要策略之一(Sultan, 1987; Alpert & Simms, 2002)。某些特定的可塑性特征可以遗传给下一代从而提高后代在恶劣条件下的生存几率(Pigliucci, 2001)。

2. 机械刺激

风力和大型食草动物的践踏是干旱地区生态系统常见的机械刺激方式。某些干旱地区生态系统中,相对较小的植被覆盖度使得植物个体间不能相互保护以抵御外界机械刺激的干扰。同为机械刺激胁迫因子,风力和践踏在强度和作用方式上存在差别。首先相对于践踏,风力对植物的作用力度相对较小;其次,风力一般作用于整个地上部分而践踏作用于一点。机械刺激一般与其他胁迫因子共同作用于植物,因此由机械刺激引起的接触形变应同时考虑与其他因子的交互影响。本研究调查了不同机械刺激因子(刷动模拟风致机械刺激和模拟践踏)与其他胁迫因子的交互作用。

模拟风致机械刺激对生长的影响

风力不仅可以直接作用于植物使其发生接触形变,还会改变植物的生存微环境

(温度, 水分蒸发和二氧化碳浓度等), 因此本研究使用机器刷动植物模拟风力的刺激作用(详见第2章)。此方法可以以相同的速度, 力度和时间刷动植物。许多研究发现机械刺激不利于植物生长(Niklas, 1998; Wang *et al.*, 2008; Anten *et al.*, 2010), 繁殖(Niklas, 1998; Cipollini, 1999)和/或者地上茎增长(Neel & Harris, 1971; Henry & Thomas, 2002); 但也有研究表明机械刺激对地上部分生长没有影响(Anten *et al.* 2005; 2009; Liu *et al.*, 2007b); 而对某些物种, 轻度机械刺激对生长具有促进作用(Michell, 1996; Biddington & Dearman, 1985; Ashby *et al.*, 1979), 机理可能为轻度机械刺激能够促进植物同化作用和细胞分裂(Yang, 2002)。第2章中, 模拟风致机械刺激增大了牛心朴子的净同化速率及生长速度。机械刺激对生长的影响可能取决于植物的类型和刺激的强度。

机械刺激与沙埋的交互作用

在流动或半流动沙地, 风力是表面沙层移动的主要驱动力。沙层移动导致的沙埋减小了植物地上部分高度以及风力作用有效叶面积。在同样风速条件下, 被沙埋植株的弯曲力矩较小。沙埋显著影响了风力对植物的机械刺激作用, 不仅直接改变了茎的弯曲曲率(Gere & Timoshenko, 1999), 也改变了风力在植物地上部分的分布。第2章发现被部分沙埋的牛心朴子在受到刷动模拟风力刺激时, 基径生长速率和茎的强度都有显著提高, 这些变化使得植物能够承受更多的地上部分重量; 而模拟风致机械刺激对未沙埋植株茎的强度没有影响。这说明刷动的机械刺激作用与沙埋间存在交互作用。对沙埋和未沙埋植株, 当弯曲相同角度, 地上高度较短的茎所需的力较大, 其可能会承受较大的压力。另一方面, 被沙埋植物的基径处较未沙埋植株基径处年幼, 而年幼组织对外界机械刺激的响应相对强烈(Biddington, 1986; Chehab *et al.*, 2009)。在干旱区生态系统, 风力强度随时间和空间而变化。低强度机械刺激造成的异速生长能减轻植物可能遭受的更强的机械刺激带来的损伤, 这对被沙埋植株尤为重要。沙埋和风致机械刺激对植物形变和机械性状有显著的交互作用(第2章), 这些塑性变化在功能上可能是相互协调的。此外, 遭受沙埋的较年幼植株在机械刺激作用下可能产生更强的可塑性响应。

不同水分条件和践踏的交互作用

在干旱和半干旱地区, 土壤水分含量是影响植物生长的重要环境因子(Hanke *et al.*, 2011)。在土壤水分含量较高环境中, 更多的生物量会被分配到植物茎进而影响其高度, 茎宽, 强度及对机械刺激的抵抗能力; 另一方面, 生物量分配的变化会影响根系的固定作用(Goodman & Ennos, 1996)。在干旱地区, 资源的分布有很强的时空异质性(Housman *et al.*, 2007), 具有不同耐旱能力的物种在异质性的环境中产生不同的可塑性响应。水分条件相对较好的斑块植被覆盖率相对较高, 其作为潜在的采食和饮水地能吸引较多的食草动物。土壤水分条件和植被遭受践踏的频率呈正相关关系。在湿润环境中, 相对较高的地上茎更易受到践踏等机械刺激的伤害, 而较粗的基径和较大的茎强度能部分补偿践踏带来的伤害。

与践踏对草本植物的作用相似(Sun & Liddle, 1993a; Andrés-Abellán *et al.*, 2006;

Hill & Pickering, 2009), 在高水分和低水分条件下, 践踏降低了四种木本植物, 柠条, 牛心朴子, 羊柴和沙棘的地上茎总长度和竖直方向高度但增大了基径的宽度(第4章)。四种木本植物的机械强度变化趋势在高水分和低水分条件下相同, 这与另一种机械刺激形式摇动对不同水分条件下羊柴的影响结果类似(Wang *et al.*, 2008)。而在同一作者的其他研究中, 草本植物虫实在不同水分条件下对机械刺激产生了不同反应, 即在低水分条件下, 机械刺激降低了虫实茎的强度但在高水条件下增大了其强度(Wang *et al.*, 2009b)。木本植物与草本植物的不同反应可能是由于其结构上的差异(Wang *et al.*, 2008; 2009b)。水分膨压在对植物对机械刺激的抵抗的机制之一, 其对草本植物的影响更大(Struik, 1965)。而对木本植物而言, 茎的组成, 如薄壁组织, 厚壁组织, 木质部, 维管组织比水分膨压作用更为重要。

践踏对克隆植物和非克隆植物的影响

大型动物对植物的践踏是干旱区生态系统的常见胁迫因子之一(Hanley & Sykes, 2009; Sørensen *et al.*, 2009; Gruntman & Novoplansky, 2011)。践踏时常与植物叶片采食同时发生。第4章研究了重复践踏对四种木本植物, 柠条, 牛心朴子, 羊柴和沙棘(每周3次, 共14周)的作用。践踏对四种木本植物的存活率没有影响, 且只降低了沙棘在两种水分条件下的生物量积累。

第3章调查了克隆植物小叶杨对践踏的耐受能力是否受克隆整合影响。毛乌素沙地生存着多种克隆木本植物, 某些物种可以在横向延伸的地下茎或根上产生分株。在干旱区沙地, 克隆植物的种群生长主要取决于其后代在沙地环境中的成功定居(Li, 2010)。践踏显著增加植物的死亡率, 且对没有与其他植株相连的克隆植物分株伤害更大, 表明克隆整合对植物耐受践踏十分重要。此外去叶处理也同时施加于小叶杨植株, 切断根茎与去叶间没有交互作用。践踏和叶片采食是放牧对植物影响的两个方面。研究结果表明克隆整合作用增大小叶杨对放牧的耐受能力主要是因为其缓解了对践踏而不是叶片采食的作用。之前很多研究表明克隆整合能够缓解遮阴(Alpert, 1999; Alpert *et al.*, 2003), 干旱(de Kroon *et al.*, 1996; Dong & Alaten, 1999), 缺肥(Alpert, 1991), 沙埋(Yu *et al.*, 2004), 风蚀(Yu *et al.*, 2008)等胁迫因子的不利影响。本研究发现了克隆整合能提高植物对践踏的耐受能力。

本研究关注的地区年降雨量较少(260–450 mm), 很多植物遭受干旱胁迫的影响。对小叶杨来说, 水平方向延伸的主根上的须根数量对于分株存活有重要意义。然后践踏能直接破坏其根系系统并阻碍根的生长。这种情况在木本植物中更严重, 因为其相对坚硬的茎在遭受机械刺激时会导致较大的弯矩、根系的移动(Crook & Ennos, 1994; van Delden *et al.*, 2010)及对剪切应力承受能力小的须根的损伤(Berry *et al.*, 2004)。践踏对根系的破坏会加重干旱对植物的胁迫作用, 这种情况也同样可能发生在非克隆木本植物上。

践踏引起的弹性和强度变化的权衡

第3章和第4章研究了茎的塑性特性变化如何影响植物的机械性状。植物有两种方法来应对外界的机械刺激, 一是增加茎的强度以增大对折断的抵抗能力, 一种

是增加弹性以减小所收到的冲击 (Puijalón *et al.* 2008; Paul-Victor & Rowe, 2011; Puijalón *et al.*, 2011)。本研究用 F_{break} 表示基径折断的最小应力 (Anten *et al.* 2005), 用 $1/F_{\text{bend}}$ 表示茎弯曲的弹性能力。其比值 $F_{\text{break}}/F_{\text{bend}}$ 可简单的用来衡量强度和弹性间的权衡关系。由于较小植株无法抵抗践踏压倒性的弯折作用, 理论上茎的弹性增大是对践踏的合理应对措施 (Sun & Liddle, 1993a)。

研究中, 总体来说践踏刺激同时增大了 F_{break} 和 F_{bend} , 但 F_{bend} 增大程度更多。这就导致了小的 $F_{\text{break}}/F_{\text{bend}}$ 比例, 即不利的弹性与强度间的权衡。这一结果与植物对其他较为温和形式的机械刺激相类似 (如风, 摩擦和摇动; Biro *et al.*, 1980; Telewski, 1990; Anten *et al.*, 2005)。植物对强度和作用方式不同的机械胁迫产生了类似的响应, 这些响应有利于增强对较温和刺激形式而非对大型动物践踏的耐受能力。

3. 去叶

世界范围内过度放牧被认为是沙漠化和草地退化的主要原因 (Squires, 2003; Yoav *et al.*, 2006; Verón & Paruelo, 2010)。放牧引起的叶片损失是放牧的主要影响之一 (McNaughton, 1983)。去叶减小了有效光合面积并影响植物的生长, 然而去叶后的补偿生长能减轻对其生长的不利影响 (McNaughton, 1983; Anten *et al.*, 2003; Gruntman & Novoplansky, 2011)。其他环境因子对能够影响植物去叶后的补偿生长能力, 如土壤养分条件的限制能够增强生长补偿能力 (Coughenour *et al.*, 1990; van Staalduin & Anten, 2005), 而遮阴则对补偿生长有不利影响 (Anten *et al.*, 2003)。本论文中研究了去叶和沙埋的交互对生长的作用。

表 1. 沙埋和去叶处理对油蒿茎高度相对生长速率 (RGR_h)、生物量相对增长速率 (RGR_m)、叶面积比 (LAR) 和净同化速率 (NAR) 影响的单因素方差分析; 以及沙埋与去叶的交互作用的双因素方差分析

	RGR_h	RGR_m	LAR	NAR
单因素方差分析				
沙埋	571.17 ^{***}	9.61 ^{***}	5.79 ^{**}	9.09 ^{***}
去叶	0.68 ^{ns}	5.62 ^{**}	19.15 ^{***}	57.30 ^{***}
双因素方差分析				
沙埋	126.62 ^{***}	0.05 ^{ns}	6.34 [*]	9.54 ^{**}
去叶	3.74 ^{ns}	1.57 ^{ns}	27.66 ^{***}	55.90 ^{***}
沙埋×去叶	2.22 ^{ns}	15.02 ^{***}	0.67 ^{ns}	0.11 ^{ns}

表中所列为 F 值和显著水平 (^{***} $P < 0.001$, ^{**} $P < 0.01$, ^{*} $P < 0.05$ and ^{ns} $P \geq 0.05$)。

对牛心朴子而言, 去叶对生长有不利影响, 与沙埋的交互作用能加重了这种作用 (第2章)。在其他的独立温室实验中 (乌德勒支大学, 合作者 Ineke Roeling), 木本植物油蒿被施加五个水平的沙埋和去叶处理 (本章图2)。单独的沙埋和去叶处理促进了植物生长速率。而先去叶后沙埋的处理降低了植物的相对生长率。这进一步

表明了沙埋对去叶的补偿生长能力有不利影响。沙埋与去叶对生长的交互作用可以

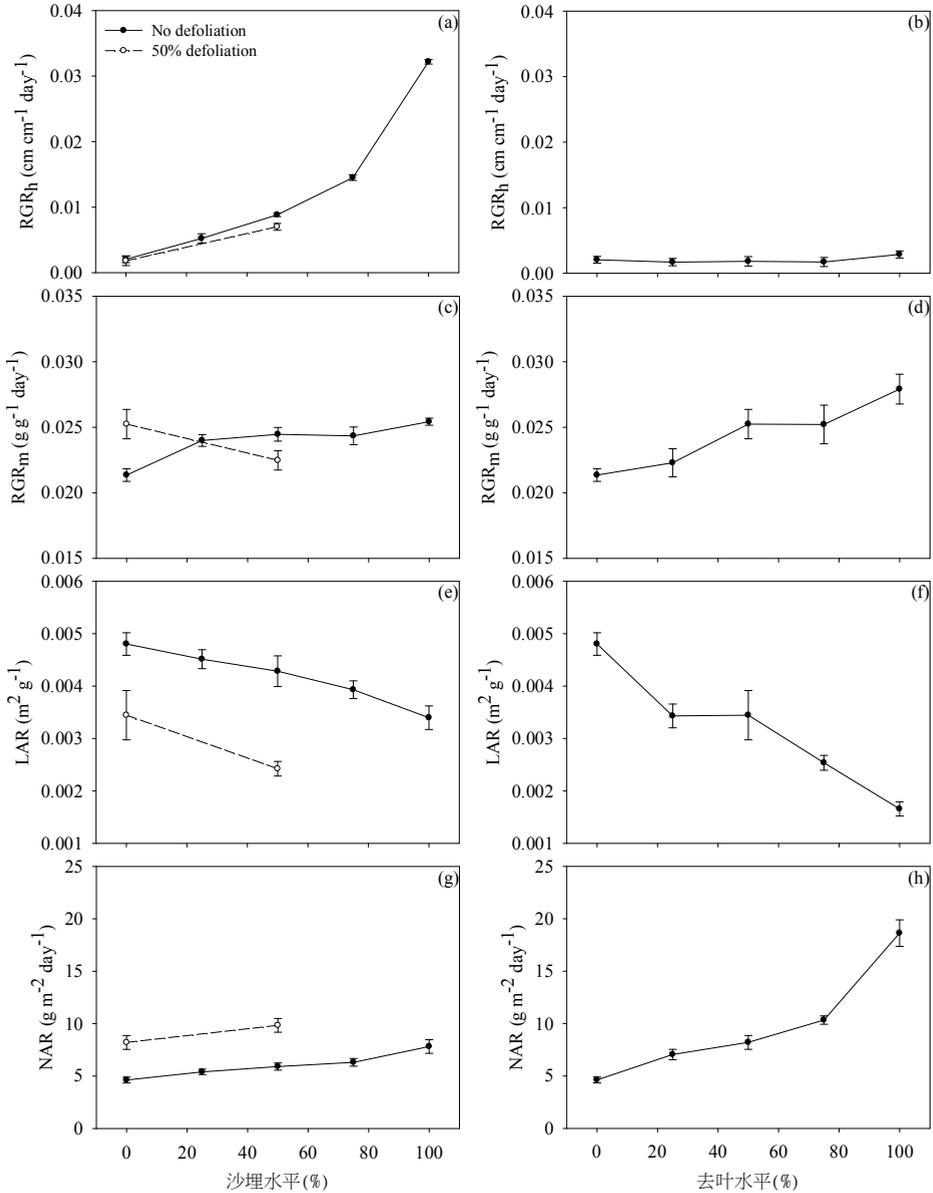


图2. 高度相对生长率(RGR_h; a, b), 生物量相对生长率(RGR_m; c, d), 叶面积比(LAR; e, f) 和净同化速率(NAR; g, h)。木本植物油蒿遭受不同水平的沙埋和去叶处理。沙埋处理, 0, 25%, 50%, 75% 和100%地上高度被掩埋。沙埋与采食的交互处理为先均匀去除50%的叶片然后再掩埋一半的地上高度。去叶处理, 0, 25%, 50%, 75% 和100%叶片被均匀去除。图a, c, e和g为沙埋及沙埋和去叶的交互处理作用。图b, d, f和h为去叶处理作用。此实验同Ineke Roeling (Utrecht University) 共同完成。

用资源限制模型 (LRM; Wise & Abrahamson, 2007) 解释。植物对采食的耐受能力受限于外界资源条件。去叶和沙埋都能够直接降低植物对外界光的获取量, 其交互作用更严重减小了植物对光的获取并加重对生长的不利影响。

4. 研究结果在沙漠化控制的应用

世界范围内草地退化和沙漠化是干旱区环境恶化的主要问题 (Squires, 2003; Yoav *et al.*, 2006; Verón & Paruelo, 2010)。本研究所关注的区域多年来遭受沙漠化威胁 (Zhang, 1994, van Staalduinen & Werger, 2006)。沙漠化过程涉及到的许多环境胁迫因子往往同时作用于沙地植被, 例如过度放牧通过去叶和践踏影响植物生长和植被覆盖, 强风作用于暴露的沙层的引起沙埋和风蚀作用等。本论文研究了环境胁迫因子对植物的影响, 特别强调这些因素的交互作用。

克隆植物被广泛应用于干旱区的沙地固定和植被恢复 (Zhang *et al.*, 2002; Yu *et al.*, 2004; Li *et al.*, 2008)。克隆植物能在水平方向延伸的根或地下茎上产生分株, 植株间可通过克隆整合分享资源, 抵抗环境胁迫因子带来的生存生长压力, 如遮阴 (Alpert, 1999; Alpert *et al.*, 2003), 干旱 (de Kroon *et al.*, 1996; Dong & Alaten, 1999), 沙埋 (Yu *et al.*, 2004), 风蚀 (Yu *et al.*, 2008) 等。本研究发现克隆整合能提高植物对放牧的耐受能力, 其对践踏的影响大于去叶。单独的践踏也可以对植物造成不利影响, 伤害随着植株大小的增大而减小。超过一定大小的植株将不再受到践踏的影响。对克隆植物而言, 较大植株可以通过克隆整合的方式补偿较小的植株所受到的伤害。从可持续性放牧管理角度, 对践踏的管理应和采食去叶放在同样重要的地位。

在沙地生态系统, 某些物种的生长依赖于沙地的流动性。例如油蒿在流动沙丘的生长状况较固定沙丘要好 (Li, 2010), 这是由于沙埋的刺激作用能够提高某些物种的生长活力 (Dech & Maun, 2006; Li, 2010)。在流动沙丘生长状况较好的非克隆植物如油蒿也可以被用于流动沙丘的固定。尽管沙埋的存在能刺激植物的生长, 但与其他胁迫因子的交互作用能对生长产生负面影响。去叶与沙埋的交互作用减小了有效光合面积和并影响植物组织间的资源再分配, 从而降低了植物的生长速率。践踏和去叶的交互作用能加重放牧对植物的影响。在流动或半固定沙丘, 放牧应被严格禁止。

在沙丘生态系统中, 一些非克隆植物对恶劣的生存环境影响有较强的耐受能力 (Li, 2010)。尽管年幼植株在生长阶段早期生长速度较慢且死亡率较高, 但度过这个阶段的植株在之后的生活阶段存活率很高 (Li, 2010)。第5章研究表明对沙埋的塑性反应可以增强植物对沙埋的耐受能力, 并帮助植物抵抗由外界机械刺激造成的损伤。可塑性变化能够在多大程度上增加植物对沙埋耐受能力还需进一步的研究。有较强耐受能力的植株可以被用来进行种子培育并用于之后的沙地固定和植被恢复。

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Curriculum vitae

Liang Xu was born on 3 September 1983 in Rushan, Shandong Province, China. He entered Central South University in Changsha, Hunan Province in 2002 and got his bachelor degree in 2006. The next step was a master degree in 2008 at Sun Yat-Sen University located in Guangzhou, Guangdong Province. In the same year, he continued his Ph.D. study on ecology in Institute of Botany, Chinese Academy of Sciences. Luckily he was selected to attend the cooperative project between Institute of Botany and Utrecht University. During the Ph.D. study, part of the research was conducted in Mu Us Sandland in Inner Mongolia, China and the other was finished at Utrecht University. The research specially focused on the plastic responses of woody species in drylands to environmental stress.

