

## Original Articles

# The antioxidation-related functional structure of plant communities: Understanding antioxidation at the plant community level



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## ABSTRACT

In the case of ecological stresses, reactive oxygen species (ROS) can be overproduced in plant cells, leading to lipid peroxidation that causes damage or death to cells. To prevent damage, plant tissues contain several antioxidants that scavenge ROS. However, antioxidation at the plant community level still remains unknown and may provide an insight into ecosystem functioning regarding stress resistance. To understand the property, we established the antioxidation-related functional structure based on the concept of the functional structure and the activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), reduced glutathione (GSH), carotenoid (Car) and proline (Pro) and the relative abundance of component species in natural plant communities on the Chinese hilly-gullied Loess Plateau. An information-theoretic (I-T) approach was used to infer the effects of antioxidation-related functional components and stresses that we investigated on lipid peroxidation at the plant community level quantified by the community-weighted mean of malondialdehyde (MDA). We found that the induction of lipid peroxidation was more closely associated with a soil available nitrogen deficiency than it was with an available phosphorus deficiency. However, the inducing effect of soil available nitrogen was finite. The prevention of lipid peroxidation was more closely associated with the community-weighted means of GSH and Pro (CWM GSH and CWM Pro) than it was with other antioxidation-related functional components. However, the efficiency of CWM Pro was quite low; CWM GSH exhibited inefficiency. In addition, antioxidation-related functional components were affected by neither soil available nitrogen nor available phosphorus. Furthermore, by estimating the relative weights of the antioxidants and considering the feasibility of community assemblages, we proposed that *Stipa grandis*, *Leymus secalinus*, *Stipa bungeana*, *Phragmites australis*, *Potentilla tanacetifolia*, *Artemisia gmelinii*, *Artemisia scoparia*, *Heteropappus altaicus* and *Syringa oblata* could be utilized in community assemblages to achieve an antioxidation-functional target. Additionally, appropriate phosphorus application for *A. gmelinii* and *H. altaicus* might contribute to maintaining their antioxidation.

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## 1. Introduction

There is often disequilibrium between pro-oxidation and anti-oxidation in plants, which is imposed by the overproduction of

reactive oxygen species (ROS) and can cause lipid peroxidation that poses disturbance to cellular metabolism, individual growth and even ecosystem functioning (Blokhina et al., 2003; Kaur et al., 2014). To prevent the disturbance, plants contain several antioxidants that scavenge ROS. Ecologically, the overproduction of ROS can be provoked by numerous abiotic and biotic stresses, such as drought, salinity, infertility, radiation, extreme temperatures, pathogen infestation, or other regimes (De Gara et al., 2003; Huang et al., 2004; Anjum et al., 2015). At present, a variety of stresses and stress interactions have been simulated to study the production of ROS, induction of lipid peroxidation, responses of antioxidants and expression of relevant genes in different plants (Keles and Oncel, 2002; Reddy et al., 2004; Herbinger et al., 2005; Shi et al., 2010;

**Abbreviations:** AN, available nitrogen; AP, available phosphorus; SM, soil moisture; ND, niche differentiation; D, dominance; NB, niche breadth; MNO, mean niche overlap; I-T, information-theoretic; MMI, multimodel inference.

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Zhang et al., 2016). However, in natural ecosystems, especially degraded ones, there are ecological stresses that are too complicated to be stimulated. In addition, the research in this field has remained at or below the level of plant individuals (De Gara et al., 2003; Myouga et al., 2008; Cartes et al., 2012). However, studying antioxidation at the plant community level should be of equal importance from the perspective of plant sociology and ecosystem functioning.

The concept of the functional structure of communities has been proposed to provide multidimensional insights into ecosystem functioning (Mouillot et al., 2011; de Bello et al., 2013). The functional structure is composed of functional identity and functional diversity. The former component is often quantified by the community-weighted mean (CWM) that represents the trait value in a community weighted by species relative abundance (Garnier et al., 2004). Functional diversity is increasingly identified as an important driver of ecosystem functioning (Hooper et al., 2005). We often quantify it based on three metrics: functional richness (FRic), reflecting the volume of functional space occupied by a community; functional divergence (FDiv), reflecting the divergence of abundance distribution in this space; and functional evenness (FEve), reflecting the regularity of abundance distribution in this space (Mason et al., 2005). At present, various aspects of ecosystem functioning—such as water use, light interception, biomass dynamics, litter decomposition, soil carbon and nitrogen storage and reduced soil erosion—have been closely associated with these functional components of plant communities based on relevant functional traits (Garnier et al., 2004; Mokany et al., 2008; Zhu et al., 2015). For example, the reduction of soil erosion could be explained by FDiv integrated by relevant root and leaf traits, CWM root diameter and CWM root tensile strength (Zhu et al., 2015). Thus, establishing the antioxidation-related functional structure of plant communities can improve the understanding of antioxidation at the plant community level and may supplement the knowledge of ecosystem functioning regarding stress resistance.

Destruction of vegetation on the Chinese Loess Plateau because of long-term human activities has resulted in severe soil erosion and ecological degradation (Zhao et al., 2015). To address this issue, the improvement of vegetation cover is critical (Zucca et al., 2015). For example, the “Grain for Green” program was initiated by Chinese government in 1999. In particular, afforestation was considered as a key technique in the past fifteen years (Li et al., 2008). However, we ignored the climatic, pedologic, hydrologic and topographic conditions that made a site unsuitable for afforestation on the one hand (Piao et al., 2010; Cao et al., 2011), and failed by introducing inappropriate species that caused more deteriorative outcomes in terms of increased soil erosion, exacerbated water shortages and deep soil desiccation on the other hand (Cao et al., 2009; Cao, 2011). Excessive afforestation and arbitrary species introduction should thus be avoided, and the use of herbaceous and native plants is wise because of their long-term adaptability (Cao et al., 2011; Jiang et al., 2013). Therefore, a prerequisite for present vegetation restoration is to study different aspects of ecosystem functioning based on plant functional traits and the functional structure of natural plant communities, especially grassland communities. According to the relationship between functional components and ecosystem functioning, functional species can be selected from the natural plant species pool to assemble functional communities, enabling us to apply these trait-based studies to practical vegetation restoration. However, the functioning of ecosystems on the Loess Plateau has not been studied from very many aspects (Zhu et al., 2015). Ecological stresses on the Loess plateau are characterized by drought, infertility, radiation, and soil erosion, all of which are often definitive control of ecosystem functioning (Li et al., 2008; Gelaw et al., 2015; de Oliveira et al., 2015; Roa-Fuentes et al., 2015; Liu et al., 2016). It should be noted

that antioxidation, reflecting long-term resistance to complicated ecological stresses, is necessary to be utilized in community assemblages to achieve an antioxidation-functional target while restoring the functioning of degraded ecosystems on the Loess Plateau.

Therefore, the objectives of our study were 1) to investigate, at the level of natural plant communities, how strongly lipid peroxidation was induced by stresses, how efficiently antioxidation-related functional components prevented lipid peroxidation and how negatively antioxidation-related functional components were affected by stresses; 2) to identify antioxidation-functional species and then select feasible species for community assemblages.

## 2. Materials and methods

### 2.1. Study area

This study was conducted in the Chenjiagua (36°49′–36°50′, 109°15′–109°19′), Wangjiagou (36°50′–36°52′, 109°10′–109°11′) and Shiziwan watersheds (37°8′–37°10′, 109°3′–109°5′), located in the hilly-gullied region of the Loess Plateau in China. Within the studied watersheds, the climate is semi-arid characterized by low annual precipitation (450–490 mm) as well as a low annual mean temperature (8–9 °C) (Li et al., 2008). According to our watershed-wide investigation, the soils are mainly silt loams (approximately 23% sand, 55% silt and 22% clay; mass percent), Calcaric Cambisols (pH values are 7.85–8.29), less compact (bulk densities are 1.11–1.32 g/cm<sup>3</sup>) and non-saline (soluble salt contents are less than 1 g/kg). The study area is distributed in the transition zone between forest and grassland. Natural shrubs are usually sparse, including *Syringa oblata*, *Sophora davidii*, *Periploca sepium*, and *Buddleja alternifolia*, among others. Natural subshrubs and perennial herbs are dominant in this area and are mainly mes-xerophytes and xerophytes, including *Artemisia gmelinii*, *Artemisia giraldii*, *Lespedeza daurica*, *Bothriochloa ischaemum*, and *Stipa bungeana*, among others.

### 2.2. Vegetation survey

Considering the fragmented landscape and common types of natural vegetation within the study area, a vegetation survey was conducted on sixteen plots (10 × 10 m) scattered on different hill-slopes that had similar altitudes, aspects, angles and species occurrences, but dissimilar species abundances (Table 1). We focused on sunny aspects for maximizing the identification of abiotic and biotic stresses and eliminating the differences in solar radiation and soil temperature. In each plot, we marked five transects (2 × 10 m) along the slope: three spaced transects were used to record vegetation information and sample plant biomass; the remaining two transects were used for soil and plant sampling. In detail, all plant species present in each transect were identified, the canopy height at maturity per species was measured, and the proportional biomass per species was sampled. The mean values of three transects were used to represent the quantitative character of each plot. Sixteen communities were defined based on the simplified importance value expressed by the species relative biomass (He et al., 2004).

### 2.3. Sampling and assays for antioxidants and malondialdehyde (MDA)

The chloroplast and mitochondrion are the main sites producing ROS. Thus, lipid peroxidation is likely to be induced in these two organelles. Hydroxyl radicals (•OH) can induce lipid peroxidation directly. Superoxide anion free radicals (O<sub>2</sub><sup>•-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and singlet oxygen (<sup>1</sup>O<sub>2</sub>) can also induce lipid peroxidation by transforming into •OH. Superoxide dismutase (SOD; EC 1.15.1.1) can catalyze O<sub>2</sub><sup>•-</sup> produced in mitochondria, chloroplasts

**Table 1**  
Vegetation and topographic information of sixteen plots.

| Plant communities   | Altitude (m) | Aspect (°) | Angle (°) |
|---|--------------|------------|-----------|
| <i>Heteropappus altaicus</i> + <i>Artemisia scoparia</i> + <i>S. bungeana</i>         | 1386         | 125        | 20        |
| <i>S. bungeana</i> + <i>L. daurica</i> + <i>Potentilla tanacetifolia</i>              | 1380         | 205        | 20        |
| <i>L. daurica</i> + <i>S. bungeana</i> + <i>H. altaicus</i> + <i>P. tanacetifolia</i> | 1251         | 195        | 22        |
| <i>Stipa grandis</i> + <i>S. bungeana</i> + <i>A. gmelinii</i>                        | 1381         | 255        | 28        |
| <i>L. daurica</i> + <i>Cleistogenes caespitosa</i>                                    | 1397         | 200        | 26        |
| <i>L. daurica</i> + <i>A. giraldii</i> + <i>Artemisia frigida</i>                     | 1301         | 120        | 28        |
| <i>L. daurica</i> + <i>P. sepium</i> + <i>B. ischaemum</i> + <i>A. gmelinii</i>       | 1275         | 190        | 23        |
| <i>A. gmelinii</i>  | 1255         | 120        | 22        |
| <i>A. gmelinii</i> + <i>L. daurica</i>  | 1367         | 240        | 20        |
| <i>P. sepium</i>  | 1268         | 105        | 26        |
| <i>P. sepium</i> + <i>A. gmelinii</i>   | 1354         | 120        | 23        |
| <i>A. gmelinii</i> + <i>P. sepium</i> + <i>A. giraldii</i> + <i>B. ischaemum</i>      | 1339         | 120        | 22        |
| <i>S. davidii</i>   | 1173         | 160        | 25        |
| <i>S. davidii</i> + <i>A. giraldii</i>  | 1199         | 170        | 27        |
| <i>S. davidii</i> + <i>P. sepium</i> + <i>A. gmelinii</i>                             | 1313         | 140        | 25        |
| <i>S. oblata</i> + <i>A. giraldii</i> + <i>A. gmelinii</i> + <i>B. ischaemum</i>      | 1248         | 230        | 28        |

and the cytoplasm. Peroxidase (POD; EC 1.11.1.7) and reduced glutathione (GSH) play important roles in the ascorbate-glutathione cycle that is the principal approach of scavenging H<sub>2</sub>O<sub>2</sub> produced in chloroplasts. GSH can also make lipid radicals (Ṙ) return to normal and scavenge lipid hydroperoxide (ROOH) in the process of lipid peroxidation. Proline (Pro) can scavenge \*OH and <sup>1</sup>O<sub>2</sub> produced in the cytoplasm (Jiang et al., 1997). Thus, GSH and Pro have direct effects in preventing lipid peroxidation. Catalase (CAT; EC 1.11.1.6) is primarily responsible for scavenging H<sub>2</sub>O<sub>2</sub> produced during photorespiration in peroxisomes. Carotenoid (Car) serves as both an inhibitor and scavenger of singlet oxygen (<sup>1</sup>O<sub>2</sub>) in chloroplasts (Zhang, 2006). Therefore, they were considered to be key antioxidants and antioxidation-related functional traits. Malondialdehyde (MDA) resulting from lipid peroxidation is a commonly used indicator of the level of lipid peroxidation (Sun and Hu, 2006).

Intact leaves of at least ten individuals per species in each plot were sampled. The sampling was fixed in the afternoon (12:00–14:00) in mid-July for the most stressful occurrence of abiotic and biotic stresses and lasted less than a week. For sample storage, we used liquid nitrogen containers in the field and an ultra-low temperature refrigerator (−80 °C) in the laboratory. All individuals per species in each plot were ground using liquid nitrogen as quickly as possible and stored in the refrigerator prior to subsequent assay. Three repetitions were conducted for every assay.

The species representing more than 85% of the cumulated biomass for each plot was selected, resulting in a list of twenty-four species for which we assayed antioxidants and MDA. The activities of SOD, POD and CAT were assayed using the nitroblue tetrazolium method (Giannopolitis and Ries, 1977), guaiacol colorimetry and H<sub>2</sub>O<sub>2</sub> ultraviolet absorption (Maehly and Chance, 1954), respectively. The contents of GSH, Car and Pro were assayed using the dithiobis-2-nitrobenzoic acid (Sun and Hu, 2006), acetone-ethyl alcohol (Zhang, 1986) and acid-ninhydrin methods (Bates et al., 1973), respectively. The content of MDA was assayed using the thiobarbituric acid method (Puckette et al., 2007).

#### 2.4. Establishing the antioxidation-related functional structure of plant communities and quantifying lipid peroxidation at the plant community level

Based on the concept of functional structure (Mouillot et al., 2011) and antioxidation-related functional trait values and the relative abundance of component species in each community, the antioxidation-related functional structure of plant communities was established. The purpose was to understand antioxidation at the plant community level that reflected long-term stress resis-

tance and was considered to be an important aspect of ecosystem functioning.

The first component of the structure was antioxidative identity, reflecting the antioxidation-related functional trait value of dominant species in a community. It was quantified using CWM. Species relative biomass was used as the species relative abundance to weight each trait value. Another component was antioxidative diversity, composed of antioxidative richness, antioxidative divergence and antioxidative evenness, reflecting the antioxidation-related functional space occupied by a community, divergence of abundance distribution in this space and regularity of abundance distribution in this space, respectively. These qualities were expressed by FRic, FDiv and FEve, respectively. The quantification of these components used the following formulas (Lavorel et al., 2008; Mason et al., 2003; Villéger et al., 2008):

$$CWM = \sum_{i=1}^S a_i x_i \quad (1)$$

$$a_i = \frac{B_i}{\sum_{i=1}^S B_i} \quad (2)$$

$$FRic = \frac{x_{\max} - x_{\min}}{AR} \quad (3)$$

$$FDiv = \frac{2}{\pi} \arctan(5V) \quad (4)$$

$$V = \sum_{i=1}^S a_i (\ln x_i - \overline{\ln x})^2 \quad (5)$$

$$\overline{\ln x} = \sum_{i=1}^S a_i \ln x_i \quad (6)$$

$$FEve = \frac{\sum_{l=1}^{S-1} \min(PEW_l, \frac{1}{S-1}) - \frac{1}{S-1}}{1 - \frac{1}{S-1}} \quad (7)$$

$$PEW_l = \frac{EW_l}{\sum_{l=1}^{S-1} EW_l} \quad (8)$$

$$EW_l = \frac{dist(i, k)}{a_i + a_k} \quad (9)$$

where  $a_i$  is the relative abundance of species  $i$  in one plot;  $B_i$  is the biomass of species  $i$  in one plot;  $n$  is the number of species in one plot;  $x_i$  is the value of one antioxidation-related functional trait of species  $i$  in one plot;  $S$  is the number of species that represented more than 85% of the cumulated biomass in one plot;  $x_{\max}$  and  $x_{\min}$  are maximum and minimum values of one trait among  $S$  species, respectively;  $AR$  is the absolute range of one trait;  $EW_l$  is weighted evenness;  $dist(i,k)$  is the Euclidean distance between species  $i$  and species  $k$ ; and the species is involved in branch  $l$ .

Meanwhile, the level of lipid peroxidation of dominant species in a community was quantified by the community-weighted mean of MDA that was calculated using Formulas (1) and (2).

To combine a set of antioxidation-related functional traits into a composite index of antioxidative diversity, we first obtained these traits on a comparable scale (Lepš et al., 2006). Each diversity index of a single trait was thus standardized by dividing it by the mean value to eliminate the effects of dimensions. Three composite values for FRic, FDiv and FEve were then obtained by averaging every six standardized diversity indices.

### 2.5. Sampling and assays for abiotic and biotic stresses

With reference to ecological stresses defined by Levitt (1980), there are not only abiotic stresses but also biotic stresses. In this study area, interspecific interaction, soil drought, alkalinity, and nitrogen and phosphorous deficiencies were considered to be the main stresses expected to influence the level of lipid peroxidation and functioning of antioxidation-related functional components and antioxidants.

Soil sampling was made using a cylinder corer (4.8 cm dia.) at the same time as plant sampling. According to our former investigation on similar slopes in the hilly-gullied region of the Loess Plateau, the maximum root biomass was distributed in the soil layer of 0–20 cm (Wang et al., 2012). Therefore, for the soil chemical measurement, we sampled to a depth of 20 cm with ten repetitive cores in each plot. However, the topsoil moisture changed constantly because of the influence of air temperature and precipitation. In addition, several dominant species in our studied communities (*L. daurica*, *P. sepium*, and *S. davidii*, etc.) were characterized by deep root systems. Most of their root biomasses were distributed in at most the soil layer of 0–80 cm (Chen et al., 2001; Wei and Shangguan, 2006; Wang et al., 2012). Therefore, for the soil moisture measurement, we sampled to a depth of 80 cm with four repetitive cores in each plot. The soil pH value (pH), available nitrogen content (AN) and available phosphorus content (AP) were determined using a pH meter (DELTA320; Mettler-Toledo International Inc., Zurich, Switzerland), Alkaline hydrolysis diffusion and an ultraviolet photometer (UV-1600; Shanghai Meipuda instrument Co., LTD, Shanghai, China) with 0.5 mol L<sup>-1</sup> NaHCO<sub>3</sub> extract, respectively (Bao, 2000). Soil moisture (SM) was determined gravimetrically in the laboratory.

For the above-mentioned twenty-four species, at least ten individuals per species in each plot were selected, and their relative young and fully expanded leaves free from pathogens and herbivores were sampled and stored in cool boxes for the specific leaf area (SLA) measurement (Cornelissen et al., 2003). The leaf area was determined using a flatbed scanner (CanoScan LiDE 210; Canon Inc., Tokyo, Japan) within 24 h after sampling and measured using image analysis software (Image-Pro PLUS 6.0, Media Cybernetics Inc., Rockville, MD, USA). We obtained the mean seed mass per species from our former study in spite of intraspecific variations (Wang, 2014). High functional divergence generally indicates a high degree of niche differentiation (Mason et al., 2005). The leaf-height-seed scheme has been proven to be a useful integrated framework for capturing key plant ecological strategies (Westoby, 1998). Therefore, the degree of niche differentiation (ND) in each

plot was estimated by the averaging the standardized values of FDiv SLA, FDiv plant height and FDiv seed mass that were calculated using Formulas (2) and (4)–(6). The dominance ( $D_i$ ), niche breadth ( $NB_i$ ) and mean niche overlap ( $MNO_i$ ) of each of twenty-four species from each plot were quantified following the formulas proposed by He et al. (2004), Hurlbert (1978) and Horn (1966):

$$D_i = \frac{300}{n} \times \frac{B_i}{\frac{1}{n} \sum_{i=1}^n B_i} \quad (10)$$

$$NB_i = \frac{\frac{1}{R} - 1}{\sum_{r=1}^R p_{ir}^2} \quad (11)$$

$$P_{ir} = \frac{D_{ir}}{N_i} \quad (12)$$

$$N_i = \sum_{r=1}^R D_{ir} \quad (13)$$

$$MNO_i = \frac{\sum_{q=1}^{n-1} NO_{iq}}{n-1} \quad (14)$$

$$NO_{iq} = \frac{2 \sum_{r=1}^R P_{ir} P_{qr}}{\sum_{r=1}^R p_{ir}^2 + \sum_{r=1}^R p_{qr}^2} \quad (15)$$

where  $n$  is the number of species in one plot;  $B_i$  is the biomass of species  $i$  in one plot;  $D_{ir}$  is the dominance of species  $i$  in resource  $r$ ;  $N_i$  is the whole dominance of species  $i$  in  $R$  resources;  $NO_{iq}$  is the niche overlap between species  $i$  and species  $q$ ; and  $MNO_i$  is the mean overlap between species  $i$  and all other coexisting species in one plot.

### 2.6. Data analysis

The among-community and interspecific variations of variables (the community-weighted mean of MDA, abiotic and biotic stresses, antioxidation-related functional components and antioxidants) were estimated by calculating the coefficients of variation (CV). The variation was considered to be large when the CV exceeded 0.25. Variables with small variations were not considered in the subsequent analyses. The values of each variable were then standardized by dividing them by the mean value to eliminate the effects of dimensions and magnitude orders without changing the variation degrees.

The effects of stresses and antioxidation-related functional components on lipid peroxidation at the plant community level were inferred using an information-theoretic (I-T) approach that made a formal inference from more than one model, i.e., multimodel inference (MMI; Burnham and Anderson, 2002). Unlike stepwise analysis arriving at a final model, this approach considered model selection uncertainty, reduced model selection bias and provided a formal relative weight of evidence for each of the alternative hypotheses. A global list of models ( $G=2^P$ ;  $P$  is the number of predictor variables) was fitted using robust regression that is an alternative to least squares regression and is not overly affected by outliers or high leverage points (Verardi and Croux, 2009). The Akaike information criterion (AIC; Akaike, 1973) was used

to compare the adequacy of these models. It rewarded descriptive accuracy via the maximum likelihood ( $\log(L_m)$ ). Models were ranked in ascending order of AIC values ( $AIC_{Cm}$ ). Models where the  $\Delta AIC_{Cm}$  (the difference between  $AIC_{Cm}$  and the minimum  $AIC_{Cm}$  of G models) was smaller than nine were considered in the confidence set (Burnham et al., 2011). Furthermore, we avoided model redundancy in the confidence set and considered a small set in which the number of models ( $g$ ) should be less than the sample size (Burnham and Anderson, 2002). R-squared values ( $R^2$ ) were used to assess the goodness-of-fit of the confidence set of models. The relative importance ( $w_{+(j)}$ ) and effect size ( $ES_{+(j)}$ ) of each predictor variable ( $x_j$ ) were quantified following the formulas described by Burnham and Anderson (2002):

$$w_{+(j)} = \sum_{m=1}^g w_m I_j(m) \quad (16)$$

$$ES_{+(j)} = \frac{\sum_{m=1}^g w_m I_j(m) \beta_{j,m}}{w_{+(j)}} \quad (17)$$

$$I_j(m) = \begin{cases} 1 & \text{if } x_j \text{ is in model } m, \\ 0 & \text{otherwise.} \end{cases} \quad (18)$$

where  $w_m$  is Akaike weight of model  $m$  interpreted as the probability that model  $m$  is the best model and  $\beta_{j,m}$  is the linear regression coefficient of predictor  $x_j$  in model  $m$ . The effects of stresses on antioxidation-related functional components were estimated using the same inference approach.

To assess the antioxidation of the above-mentioned twenty-four species for assembling communities that minimize lipid peroxidation, antioxidants that had large differences in contents among these species were considered to be assessment indices and given different weights. The weight of each antioxidant ( $W_j$ ) and antioxidation of each species ( $Ao_i$ ) were quantified following the formulas:

$$W_j = w_{+(j)} \times (-ES_{+(j)}) \quad (19)$$

$$Ao_i = \sum_{j=1}^p W_j \times sx_{j,i} \quad (20)$$

where  $p$  is the number of antioxidants considered assessment indices and  $sx_{j,i}$  is the standardized mean value of antioxidant  $j$  of species  $i$  in all of the present plots. The larger the  $Ao_i$ , the more antioxidation-functional species  $i$  is. Furthermore, the effects of stresses on heavyweight antioxidants per antioxidation-functional species were estimated using the same inference approach.

According to relevant literature, species that were widespread in the hilly-gullied region of the Loess Plateau and had large seed production and large percentages of seed germination were selected from antioxidation-functional species and considered for use in community assemblages.

All analyses were performed using Stata 12.0 (StataCorp LP, College Station, Texas, USA).

### 3. Results

Among sixteen communities, eight variables (FRic, FEve, CWMs of POD, CAT, GSH and Pro, soil available nitrogen and available phosphorus) varied largely and were thus used to predict the large variation of the community-weighted mean of MDA, while other variables (FDiv, the CWMs of SOD and Car, soil moisture, soil pH values and niche differentiation) varied little and were not considered to be predictor variables (Table 2). The confidence set of prediction models contained fourteen models. More than one predictor variable was included in each model, indicating that the community-weighted mean of MDA could not be determined by a single predictor. Most models had high R-squared values, exhibiting a strong power of prediction. In particular, the top model had a high probability of being the best model and large R-squared value, which presented substantial empirical support (Table 3).

CWM GSH was included in all of the models, so it was the most important. In addition, the magnitude and direction of its effect size were large and positive, respectively. CWM Pro and soil available nitrogen were rare in the model set, but they were included in the top model that had a high probability, so they were important as well. However, their effect sizes did not match their relative importance. They were negatively correlated with MDA. CWM CAT, CWM POD, FEve, soil available phosphorus and FRic were of little importance and had small effects (Fig. 1). In addition, soil available nitrogen and available phosphorus exhibited very low R-squared values in predicting antioxidation-related functional components (Table 4).

Among twenty-four species, POD, CAT, GSH and Pro differed greatly and were thus used to assess the antioxidation of these species, while SOD and Car differed little, so they were not considered to be assessment indices. According to the values of  $W_j$ , GSH carried considerable weight relative to POD, CAT and Pro. According to the values of  $Ao_i$ , *S. grandis* was the most antioxidation-functional. The remaining species were antioxidation-functional as well, except for *L. daurica*, *Buddleja alternifolia*, *B. ischaemum*, *P. sepium*, *C. chinensis* and *A. giraldii* (Table 5).

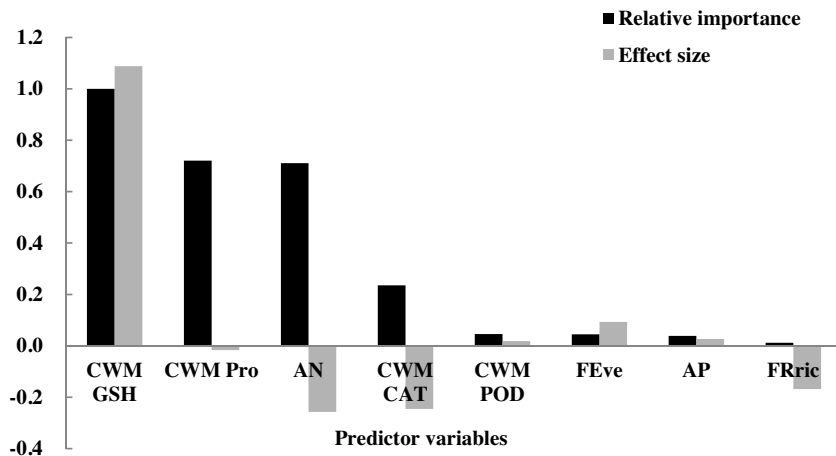
According to the direction of the weight of GSH, stresses that were negatively correlated with GSH would not contribute to minimizing lipid peroxidation as they intensified. For *S. bungeana*, soil

**Table 2**  
Coefficients of variation (CV) of the community-weighted mean of MDA, antioxidation-related functional components and stresses among sixteen communities.

| Context   | Indices                            | CV           |
|---|------------------------------------|--------------|
| Lipid peroxidation at the plant community level | The community-weighted mean of MDA | <b>0.382</b> |
|   | FRic                               | <b>0.492</b> |
|   | FDiv                               | 0.112        |
|   | FEve                               | <b>0.289</b> |
| Antioxidation-related functional components     | CWM SOD                            | 0.137        |
|   | CWM POD                            | <b>1.136</b> |
|   | CWM CAT                            | <b>0.735</b> |
|   | CWM GSH                            | <b>0.269</b> |
|   | CWM Car                            | 0.152        |
|   | CWM Pro                            | <b>0.430</b> |
|   | SM                                 | 0.132        |
| Stresses  | pH                                 | 0.016        |
|   | Abiotic stresses                   | <b>0.389</b> |
|   | AN                                 | <b>0.367</b> |
|   | AP                                 | <b>0.367</b> |
|   | Biotic stress                      | 0.095        |
|   | ND                                 | 0.095        |

**Table 3**  
The confidence set of prediction models of the community-weighted mean of MDA.

| FRric  | $\beta_{j,m}$ of predictor variables |         |         |         |         |        |       | $\log(L_m)$ | AIC <sub>Cm</sub> | $\Delta$ AIC <sub>Cm</sub> | $w_m$        | $R^2$        |
|--------|--------------------------------------|---------|---------|---------|---------|--------|-------|-------------|-------------------|----------------------------|--------------|--------------|
|        | FEve                                 | CWM POD | CWM CAT | CWM GSH | CWM Pro | AN     | AP    |             |                   |                            |              |              |
|        |                                      |         |         | 1.231   | -0.011  | -0.261 |       | 27.0        | -42.3             | 0.0                        | <b>0.663</b> | <b>0.623</b> |
|        |                                      |         | -0.237  | 0.650   |         |        |       | 23.4        | -38.7             | 3.6                        | 0.111        | 0.829        |
|        |                                      |         | -0.270  | 0.767   | -0.136  |        |       | 23.9        | -36.2             | 6.1                        | 0.031        | 0.859        |
|        |                                      | 0.026   | -0.237  | 0.691   |         |        |       | 23.8        | -35.9             | 6.4                        | 0.027        | 0.854        |
|        | 0.041                                |         | -0.250  | 0.635   |         |        |       | 23.7        | -35.7             | 6.6                        | 0.024        | 0.841        |
|        |                                      |         |         | 1.222   |         | -0.253 |       | 21.7        | -35.4             | 6.8                        | 0.022        | 0.629        |
|        |                                      |         | -0.249  | 0.656   |         |        | 0.038 | 23.4        | -35.3             | 7.0                        | 0.020        | 0.841        |
|        |                                      |         |         | 1.053   |         |        | 0.015 | 21.6        | -35.2             | 7.1                        | 0.019        | 0.477        |
|        |                                      | 0.007   |         | 1.070   |         |        |       | 21.6        | -35.1             | 7.2                        | 0.019        | 0.478        |
|        |                                      |         |         | 1.062   | 0.057   |        |       | 21.6        | -35.1             | 7.2                        | 0.018        | 0.473        |
|        |                                      |         | -0.246  | 0.735   |         | -0.124 |       | 23.1        | -34.6             | 7.7                        | 0.014        | 0.949        |
|        | 0.194                                |         |         | 1.140   |         | -0.233 |       | 23.0        | -34.3             | 8.0                        | 0.012        | 0.548        |
| -0.169 |                                      |         |         | 1.014   |         |        |       | 21.1        | -34.3             | 8.0                        | 0.012        | 0.606        |
|        | 0.095                                |         | -0.287  | 0.802   | -0.153  |        |       | 24.8        | -33.6             | 8.7                        | 0.009        | 0.842        |



**Fig. 1.** Relative importance and effect sizes of the predictor variables of the community-weighted mean of MDA.

**Table 4**  
R-squared values ( $R^2$ ) of soil available nitrogen and available phosphorus for predicting antioxidation-related functional components.

| Antioxidation-related functional components | Predictor variables | $R^2$ |
|---|---------------------|-------|
| FRic  | AN                  | 0.044 |
|   | AP                  | 0.016 |
|   | AN & AP             | 0.122 |
| FEve  | AN                  | 0.040 |
|   | AP                  | 0.196 |
|   | AN & AP             | 0.174 |
| CWM POD                                     | AN                  | 0.084 |
|   | AP                  | 0.008 |
|   | AN & AP             | 0.091 |
| CWM CAT                                     | AN                  | 0.179 |
|   | AP                  | 0.002 |
|   | AN & AP             | 0.246 |
| CWM GSH                                     | AN                  | 0.050 |
|   | AP                  | 0.067 |
|   | AN & AP             | 0.174 |
| CWM Pro                                     | AN                  | 0.011 |
|   | AP                  | 0.009 |
|   | AN & AP             | 0.010 |

available phosphorus and its dominance were the most important predictor variables of GSH and were negatively correlated with GSH, but their inducing effects on GSH were small. For *A. gmelinii* and *H. altaicus*, soil available phosphorus was the most important

predictor variable of GSH and greatly induced GSH as it became deficient (Fig. 2).

#### 4. Discussion

##### 4.1. Antioxidation at the plant community level

Numerous abiotic and biotic stresses can cause overproduction of ROS in plant cells. ROS accumulation tends to cause lipid peroxidation, giving way to oxidative degradation of lipids and formation of lipoperoxides, resulting in cellular damage or cell death (Anjum et al., 2015). In our study, lipid peroxidation varied largely among communities. We found that its variation was closely associated with soil available nitrogen while available phosphorus appeared insignificant, according to their relative importance. Soil drought and alkalinity were even worse than available phosphorus because of their small variations among communities. However, the inducing effect of soil available nitrogen on lipid peroxidation was finite, according to its relative small effect size. There may be latent stresses that have both relative great importance and relative large effect sizes; for example, soil erosion and plant pathogens were included in abiotic stresses and biotic stresses, respectively (Levitt 1980; Atkinson and Urwin, 2012).

Furthermore, we found that the variation of lipid peroxidation among communities was more closely associated with the antioxidative identity than it was with the antioxidative diversity, according to their relative importance. This result seems to contradict many studies that have confirmed the importance of

**Table 5**  
Antioxidation assessment of twenty-four species.

| Species                         | $sx_{j,i}$ of antioxidants |              |              |              |       |       | $Ao_i$ |
|---------------------------------|----------------------------|--------------|--------------|--------------|-------|-------|--------|
|                                 | POD                        | CAT          | GSH          | Pro          | SOD   | Car   |        |
| <i>S. grandis</i>               | 1.12                       | 1.60         | 0.51         | 0.39         | 0.51  | 1.00  | −0.46  |
| <i>Leymus secalinus</i>         | 1.36                       | 3.33         | 0.67         | 0.81         | 0.95  | 0.84  | −0.53  |
| <i>S. bungeana</i>              | 2.18                       | 4.31         | 0.75         | 0.40         | 1.07  | 1.08  | −0.56  |
| <i>Thymus mongolicus</i>        | 0.00                       | 0.04         | 0.57         | 0.20         | 1.19  | 0.86  | −0.61  |
| <i>Wikstroemia chamaedaphne</i> | 0.17                       | 0.69         | 0.75         | 10.16        | 0.27  | 0.92  | −0.65  |
| <i>Phragmites australis</i>     | 0.06                       | 0.30         | 0.63         | 0.60         | 0.64  | 1.06  | −0.66  |
| <i>Astragalus scaberimus</i>    | 5.14                       | 3.91         | 0.86         | 1.01         | 1.24  | 0.69  | −0.71  |
| <i>A. frigida</i>               | 0.01                       | 0.31         | 0.70         | 0.49         | 1.19  | 0.79  | −0.74  |
| <i>Astragalus discolor</i>      | 8.68                       | 3.37         | 0.91         | 1.01         | 1.11  | 0.72  | −0.79  |
| <i>S. davidii</i>               | 2.32                       | 0.44         | 0.84         | 1.23         | 1.20  | 1.12  | −0.88  |
| <i>Glycyrrhiza uralensis</i>    | 0.00                       | 0.12         | 0.84         | 0.85         | 1.17  | 0.79  | −0.90  |
| <i>P. tanacetifolia</i>         | 0.00                       | 0.34         | 0.87         | 0.29         | 0.97  | 1.23  | −0.93  |
| <i>Astragalus melilotoides</i>  | 1.12                       | 1.08         | 0.95         | 0.47         | 1.16  | 1.27  | −0.96  |
| <i>A. gmelinii</i>              | 0.04                       | 0.22         | 1.00         | 0.60         | 0.98  | 0.90  | −1.07  |
| <i>A. scoparia</i>              | 0.02                       | 0.26         | 1.01         | 0.84         | 1.01  | 0.85  | −1.07  |
| <i>Cleistogenes squarrosa</i>   | 0.66                       | 0.45         | 1.02         | 0.64         | 0.85  | 1.38  | −1.08  |
| <i>H. altaicus</i>              | 0.07                       | 0.48         | 1.04         | 0.42         | 1.19  | 0.93  | −1.10  |
| <i>S. oblata</i>                | 0.14                       | 0.37         | 1.07         | 0.22         | 0.93  | 1.05  | −1.14  |
| <i>L. daurica</i>               | 0.00                       | 0.17         | 1.22         | 0.47         | 1.11  | 1.24  | −1.31  |
| <i>Buddleja alternifolia</i>    | 0.05                       | 0.37         | 1.31         | 0.67         | 1.11  | 1.05  | −1.39  |
| <i>B. ischaemum</i>             | 0.16                       | 0.16         | 1.32         | 0.54         | 1.23  | 1.31  | −1.42  |
| <i>P. sepium</i>                | 0.18                       | 0.29         | 1.37         | 0.53         | 1.11  | 0.82  | −1.47  |
| <i>C. caespitosa</i>            | 0.46                       | 0.99         | 1.60         | 0.50         | 1.12  | 1.29  | −1.67  |
| <i>A. giraldii</i>              | 0.06                       | 0.41         | 2.18         | 0.66         | 0.69  | 0.81  | −2.34  |
| CV                              | <b>2.010</b>               | <b>1.305</b> | <b>0.368</b> | <b>1.968</b> | 0.248 | 0.204 | –      |
| $W_j$                           | −0.001                     | 0.058        | −1.088       | 0.012        | –     | –     | –      |

functional diversity to ecosystem functioning, such as productivity, resilience to invasion and reducing soil erosion, based on other functional traits (Dukes, 2001; Hooper and Dukes, 2004; Zhu et al., 2015). However, a lack of clarity remains on which traits of functional diversity are actually effect traits (Naem and Wright, 2003). In addition, this result is more indicative of the hypothesis that functional identity is more important than functional diversity in influencing ecosystem processes (Mokany et al., 2008; Laughlin, 2011). More precisely, the variation of lipid peroxidation was closely associated with CWM GSH and CWM Pro, while CWM CAT and CWM POD appeared insignificant, according to their relative importance. CWM SOD and CWM Car were even worse than CWM CAT and CWM POD because of their small variations among communities. This result may be because GSH and Pro are able to prevent lipid peroxidation directly, while other antioxidants have indirect effects in preventing lipid peroxidation. Moreover, MDA can damage protein, decreasing the activities of antioxidative enzymes (Zhang, 2006), which might neutralize the importance of CAT, POD and SOD.

We were more concerned with the efficiency of CWM GSH and CWM Pro in preventing lipid peroxidation. In the case of ecological stresses, higher activities of antioxidants are supposed to be better for counterbalancing the overproduction of ROS and thus preventing lipid peroxidation. However, we found that the efficiency of CWM Pro in preventing lipid peroxidation was quite low, according to its small effect size. Even if the effect size of CWM GSH was large, it prevented lipid peroxidation inefficiently according to the direction of its effect. This result seems incomprehensible. However, the limitation of these two antioxidants should be acknowledged. Actually, the main role of GSH in scavenging  $H_2O_2$  is as an assistant of antioxidative enzymes in the ascorbate-glutathione cycle (Zhang, 2006); Pro is specific to  $\bullet OH$  and  $^1O_2$  but has no scavenging effect on  $O_2\bullet^-$  and  $H_2O_2$  (Jiang et al., 1997).

Furthermore, we inferred how negatively stresses affected these antioxidation-related functional components. The soil in this study area lacks available nitrogen and available phosphorus to varying degrees. Nitrogen deficiency can impose restrictions on the synthesis of nitrogenous compounds in plants that lead to

decreased activities of antioxidants. Phosphorus deficiency can pose disturbance to oxidative phosphorylation in mitochondria and photosynthetic phosphorylation in chloroplasts, leading to the production of ROS. In particular,  $\bullet OH$  and  $O_2\bullet^-$  can damage protein, so they inactivate antioxidants that are unable to scavenge them (Zhang, 2006). However, our study was conducted at the scale of soil properties. At this fine scale, environmental filters significantly affect species abundance and dominance, rather than species selection and trait range (Woodward and Diament, 1991; Weiher and Keddy, 1995; Díaz et al., 1998). In particular, the vegetation among our plots primarily differed in species relative abundance. Thus, the variation of antioxidation-related functional components might greatly depend on the variation of species relative abundance. In other words, the effects of stresses on antioxidation-related functional components mostly arrived at the species relative abundance rather than the activities of antioxidants. In our study, antioxidation-related functional components were driven by neither soil available nitrogen nor available phosphorus, according to their low R-squared values.

Minimization of stress-induced damage and enhancement of resistance is generally thought to have fitness costs in terms of reduced growth (Larcher, 1997). However, the trade-off between resistance and growth has always been controversial, demonstrating that it is either present or absent in different ecosystems (Sierra-Almeida et al., 2010; Molina-Montenegro et al., 2012). Lower values of SLA usually correspond with relatively high investments in leaf defense (Cornelissen et al., 2003). An important trade-off also occur between plant height and avoidance of environmental stresses (Perez-Harguindeguy et al., 2013). In our study, the correlations of CWM SLA and CWM plant height with the level of lipid peroxidation were too loose (Pearson Correlations were 0.453 and 0.264, respectively), supporting the view on non-trade-off.

#### 4.2. Application to vegetation restoration

A trait-based restoration framework has been proposed to achieve functional goals for restoring the functioning of degraded ecosystems (Laughlin, 2014; Zhu et al., 2015). Artificially, we can

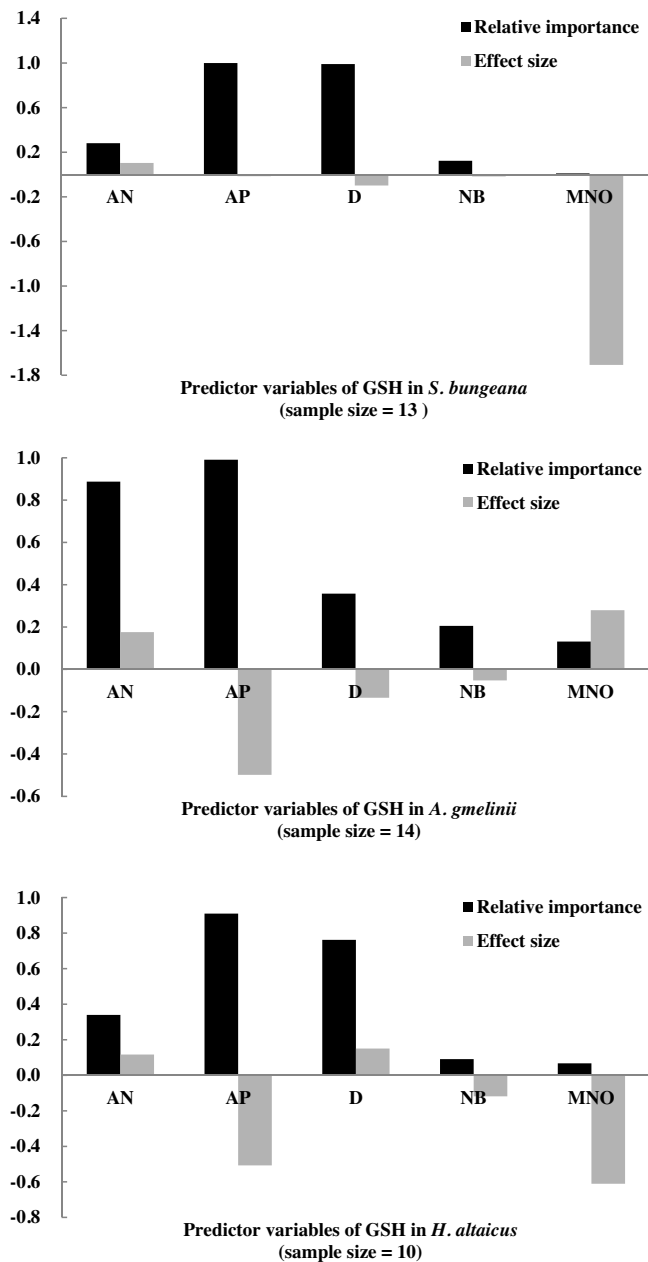


Fig. 2. Relative importance and effect sizes of the predictor variables of GSH at the species level.

select functional plant species and assemble functional plant communities by investigating the rules of different aspects of ecosystem functioning.

Antioxidation reflects long-term stress resistance and should be considered to be an important aspect of ecosystem functioning. Therefore, we attempted to achieve an antioxidation-functional goal while assembling communities. Antioxidation-functional species were preferred. After inferring antioxidation at the community level, different antioxidants were given different weights of different magnitudes and directions to assess the antioxidation of twenty-four species. To obtain a more reliable result, we probably need to study other antioxidants, such as dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR) and ascorbic acid (ASA) (Zhang, 2006).

Except for being antioxidation-functional, species that can be established easily might be more important. In other words, we should consider how extensively these species can adapt, how eas-

ily we can obtain their seeds and how likely seeds can germinate after sowing. Among the antioxidation-functional species identified in our study, *T. mongolicus*, *W. chamaedaphne* and *A. frigida* were not widespread; *A. scaberrimus*, *A. discolor* and *C. squarrosa* had very finite seed production; the seeds of *S. davidii*, *G. uralensis* and *A. melilotoides* had very low germination percentages because of their hardness. Therefore, they were not feasible for practical assemblages. Conversely, *S. grandis*, *L. secalinus*, *S. bungeana*, *P. australis*, *P. tanacetifolia*, *A. gmelinii*, *A. scoparia*, *H. altaicus* and *S. oblata* were feasible species for achieving an antioxidation-functional target because of their wide distribution, large seed production (100–5000 seeds per plant) and large percentages of seed germination (40–90%) (Wang, 2014).

Furthermore, to restore the functioning of degraded ecosystems in this study area, achieving an antioxidation-functional target is not enough. Thus, research on other important aspects of ecosystem functioning is urgently needed, for example, the storage and cycling of soil organic carbon and total nitrogen (Singh et al., 2016). Moreover, these nine antioxidation-functional species may not be superior in terms of other aspects of ecosystem functioning. Thus, repeated screening of species is necessary. Then, the trade-offs among different aspects of ecosystem functioning and between functional identity and functional diversity should be seriously considered to allocate the relative abundance of each species. To attain the optimal assemblage patterns, more than 5000 simulations should be run (Dias et al., 2013). Pilot experiments should then be carried out to validate the feasibility of the selected assemblage patterns (Zhu et al., 2015). Additionally, a proper selection of tillage methods is very important when establishing communities (de Moraes Sá et al., 2015). After establishment, the maintenance of their functional composition is challenging, considering the presence of the original soil seed bank and vegetative legacies, effects of the surrounding vegetation, and necessity of nutrient management (Zhang et al., 2015; Shi et al., 2016). For example, we found that the deficiency of soil available phosphorus could not contribute to minimizing lipid peroxidation of *A. gmelinii* and *H. altaicus*; appropriate application of phosphorus fertilizer in community assemblages should thus be necessary.

## 5. Conclusions

At the plant community level, the induction of lipid peroxidation was more closely associated with a soil available nitrogen deficiency than it was with an available phosphorus deficiency. However, the inducing effect of soil available nitrogen on lipid peroxidation was finite. The prevention of lipid peroxidation was more closely associated with CWM GSH and CWM Pro than it was with other antioxidation-functional components. However, the efficiency of CWM Pro and CWM GSH in preventing lipid peroxidation was unsatisfactory. Furthermore, antioxidation-related functional components were affected by neither soil available nitrogen nor available phosphorus.

*S. grandis*, *L. secalinus*, *S. bungeana*, *P. australis*, *P. tanacetifolia*, *A. gmelinii*, *A. scoparia*, *H. altaicus* and *S. oblata* could be utilized in community assemblages to achieve an antioxidation-functional target. In addition, appropriate phosphorus application for *A. gmelinii* and *H. altaicus* might contribute to maintaining their antioxidation.

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