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Trace element composition of tree fodder and potential nutritional use for livestock

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Short title: Tree fodder for Livestock

Highlights

- Zinc and cobalt concentrations of willow leaves were multi-fold higher than sheep requirements (83)
- Leaf selenium concentrations were more dependent on site than tree species (74)
- Metabolisable energy was appropriate to support lamb growth in all species but alder was the highest (85)
- Leaf crude protein was higher in spring but should support lamb growth in autumn to (86)

Abstract (400 words)

Silvopastoral agroforestry, the integration of trees into livestock production systems, is an ancient practice with benefits to animal welfare and nutrition. Intensification of farming practices have reduced the presence of trees and hedgerows in the agricultural landscape. Environmental benefits coupled with improvements to ecological resilience and the long-term sustainability of farm productivity have led to a resurgence in interest in silvopastoral farming systems. The objective of this study was to investigate the nutritional composition and potential use of tree leaves as a supplementary fodder for ruminant livestock, with particular reference to sheep.

Leaves (including petioles) were collected during spring (June) and autumn (September) from goat willow (*Salix caprea*), oak (*Quercus* spp) and alder (*Alnus* spp) from three sites in the UK. On the third site samples of ash (*Fraxinus excelsior*), beech (*Fagus sylvatica*), sweet chestnut (*Castenea sativa*) and sycamore (*Acer*

pseudoplatanus) were also collected. Tree leaves were analysed to determine mineral content, dry matter (DM), crude protein (CP), modified acid detergent fibre (MADF) and metabolisable energy (ME) which were then compared to the nutritional requirements of grazing sheep (*Ovis aries*).

Leaves from all tree species used in this study were able to exceed the dietary ME and CP concentration requirements (NRC) for growing lambs (40 kg lamb @ 150 g/d). Alder contained the most ME and CP of the studied species. There was no significant effect of season although CP was higher in spring than autumn for all tree species.

Zinc and cobalt concentrations were found to be dependent on tree species with negligible site and season effects. All (NRC) sheep requirements of both elements were exceeded by willow, met by alder and not met by oak, willow exceeded these requirements for zinc and cobalt by approximately 3-6 and 10-15 fold respectively. Leaf selenium concentrations were site specific with site 1 almost able to meet maximal requirement, whilst all other sites (all tree species) were around the minimal requirement.

To conclude, ME and CP concentrations of the tree leaves were generally within a requirement range to support adequate growth of lambs if leaves fed alone (not likely in practice). Selenium concentrations were site dependant, iodine was mainly season dependent with tree species effects for zinc and cobalt. The zinc and especially cobalt concentrations of willow leaves were sufficient to suggest that willow could be used as a bio-supplement when fed within a conventional grazing system, especially useful for growing lambs.

Keywords: agroforestry, silvopasture, minerals, supplementary feeding, grazing, browse.

1. Introduction

Trees have traditionally been important elements of agricultural systems around the world, evolving from systems of shifting cultivation towards settled systems integrated with agriculture, woodland grazing and silvopasture (trees and livestock), with fertility transfer from woodlands to cultivated land via manure (Eichhorn et al., 2006; Von Maydell, 1995). In silvopastoral systems, trees offer two main advantages: First, trees modify microclimatic conditions that include solar radiation, temperature, humidity, and wind speed, which can have beneficial effects on pasture growth and animal welfare (Bird, 1998; Jose et al., 2004). Second, trees can provide alternative sources of nutrients. Browse from trees and shrubs plays an important role in feeding ruminants in many parts of the world, particularly in the tropics, where there has been considerable research into the nutritional potential and limitations of many tropical fodder species (Devendra, 1992). In northern European countries, the role of trees in providing shelter is well established (He et al., 2017). However, the potential value of tree fodder as a supplement to dietary requirements and buffering against climate extremes that influence the availability of animal feed (e.g. pasture productivity and silage production) is poorly understood. Comparatively little is known about the potential of temperate browse species although the evidence base is slowly growing (Emile et al., 2016; Smith et al., 2012) with data being collated in an on-line database of nutritional values (Luske et al., 2017).

Fodder from some tree species compare favourably with typical forages such as hay, grass silage and grazed grass (Ministry of Agriculture Fisheries and Food, 1990) in terms of the major nutrient composition (energy and protein). Of greater value, however, may be their potential as a source of minerals. For example, willow (*Salix* spp.) leaves contain high concentrations of magnesium and zinc (Robinson et al. 2005). Secondary compounds such as condensed tannins may also be of benefit to ruminants by increasing the flow of rumen-bypass protein and essential amino acids to the small intestine, preventing bloat, providing anti-parasitic effects and lowering emissions of ammonia and methane (Rogosic et al., 2006; Mueller-Harvey, 2006). The potential for self-medication in ruminants is not yet well explained in the scientific literature, although salicin, in willow, is well known to have antiinflammatory and mild antimicrobial properties. However, it has not been widely evaluated in terms of its content within tree fodder or consequent effects on animal health (Boeckler et al., 2011).

Trees provide alternative feed resources during periods of low forage availability. In northern temperate systems, this role may increase in importance as the effects of climate change impact on plant growth patterns. There is also potential for preserved tree fodder to fill the 'hungry gap' in early spring, before the new season grass is available, (e.g. by drying as 'tree hay' (Green, 2016), or being ensiled (Smith et al., 2014)).

Browsing trees has historically been practised extensively throughout the UK, but the practice appears to have largely died out. Traditionally, many species of deciduous tree have been used for fodder, in particular wych or scots elm (*Ulmus glabra*), ash (*Fraxinus excelsior*), silver birch (*Betula pendula*), downy birch (*Betula pubescens*) and goat willow (*Salix caprea*) (Austad and Hauge, 2006). Goat willow is thought to be so named due to the use of the tree as a browse source for goats, and has traditionally been used as a fodder for livestock (Austad and Hauge 2006) with organic matter digestibility similar to hay and grass silage (Musonda et al., 2009; Pitta et al., 2007). One of the limitations of using tree fodder as a feed is that nutritive value and digestibility peaks in spring and decreases through to autumn (McWilliam et al., 2005).

	typical pasture		requirement*
	range	average	range
	mg/kg DM	mg/kg DM	mg/kg DM
copper	2-15	8	3-10
cobalt	0.05-0.25	0.1	0.1-0.2
selenium	0.02-0.15	0.07	0.04-0.48
iodine	0.1-0.5	0.15	0.5-0.8
manganese	25-250	100	10-34
zinc	20-60	50	21-46

Table 1 Comparison of grazing composition (range and average) of typical UK pasture (Peers and Phillips, 2011) against sheep requirements (*calculated from NRC, 2007) for the key trace elements.

Trees are often utilised as an emergency forage source (e.g. during drought) but are rarely incorporated into routine grazing management. To fully integrate and utilise the browsing/forage potential of tree leaves, the leaves need to have a nutritional composition comparable to grazed swards: Crude protein (CP) content is likely to vary from 190 g/kg DM down to 100 g/kg DM for fresh young ryegrass pastures to old mature grass pastures. Metabolisable energy (ME) will similarly range from 11 to 8 MJ ME/kg DM (McDonald et al, 2011). The typical pasture composition of the nutritionally relevant trace elements to grazing ruminants is summarised within Table 1, alongside the requirement range (calculated from NRC, 2007). Copper is a complicated element due to the availability of copper being affected by two key interactions (iron-sulphur-copper and molybdenum-sulphur-copper) with the second interaction in the absence of rumen available copper allowing thiomolybdates into the animal caused by binding with copper systemically (Gould and Kendall, 2011). The other elements are a little simpler and a grazing content unable to fulfil the published requirement will result in a loss of production and ultimately clinical signs of mineral imbalance (in this case deficiency). Growing lambs have the highest cobalt requirement (0.2 mg/kg DM, NRC, 2007) and deficiency of cobalt in the summer/early autumn period is one of the major trace element issues in grazing sheep. This is exacerbated by the seasonality of cobalt concentrations within the grass where the nadir in cobalt (Aug/Sept) matches a period in which productive lambs are required to have a good growth rate (Kendall et al., 2017).

The European browse database (Luske et al., 2017) has limitations in some of the data presented. There are CP ranges for species of alder ranging from 14-26 % DM, with a tendency for CP content to decline from spring to autumn. There is much more limited data for oak and willow and it is sometimes unclear what sample type is being presented (eg leaf only or leaf and small twig). The database does not include any values for the ME content and some of the trace elements are not appropriately rounded; for example the cobalt and selenium concentrations are quoted as either 0 or 1 mg/kg DM where they need to be quoted to at least 1 if not 2 decimal places to be able to use the data as, for example, the lamb cobalt requirement is 0.2 mg.kg DM (NRC, 2007).

Therefore, there is still a need to define key macro and mineral nutrition parameters of tree fodder in the UK in a robust manner across multiple sites and through the grazing season. Other benefits of incorporating/utilising trees within grazing systems are not explored in detail in this paper but include the effect of trees on the grass under trees though changes in microclimate (Devkota et al., 2009), and other ecosystem functions e.g. nutrient cycling, hydrological regulation (Sollen-Norrlin et al., 2020) as well as the more holistic biodiversity, connectivity and carbon sequestration.

Aim: to investigate the nutritional composition of tree fodder and estimate the supplementary potential for livestock.

Objective 1: determine composition of leaves in terms of trace elements important within sheep grazing systems and the effect of tree species, site and time during the growing season.

Objective 2: determine composition of energy and protein in the various tree species leaves over different sites and at different times during the growing season.

Objective 3: Evaluate the potential use of tree fodder as a supplementary food source for livestock.

2. Materials and methods

2.1 Site descriptions

This work was carried out across three sites spread across the UK. These were:

Organic Research Centre, Elm Farm (site 1), a 85ha organic livestock farm in West Berkshire (51°23’14.19”N; 1°24’08.34”W). Soils are classified as a Eutric Luvic

Planosols (Michéli et al., 2006) with soil types varying from heavy clay loam to sandy loam (Wickham series). Sampled trees were spread across the farm and either in boundary hedges or in-field.

The Allerton Project (site 2) is a 333ha mixed arable and livestock farm in Leicestershire (52°36'31.48"N; 0°50'1.67"W) with undulating topography around 150m asl. Soils are classified as Calcaric Stagnic Cambisol (Michéli et al., 2006) and are mainly Hanslope, Denchworth, Ragdale and Oxpasture clays. Sampled trees were in-field and distributed across the farm.

The third main and an additional fourth site were located at Bangor University's research field station located at Abergwyngreygn, North Wales (53°14'16"N, 4°01'1"W). Sampled trees were located in two adjacent fields that comprise the Bangor Diverse tree biodiversity and ecosystem function infrastructure (site 3) (Smith et al., 2013; Ahmed et al., 2016), and the silvopastoral agroforestry platform (site 4) (Teklehaimanot and Sinclair, 1993). Soils are classified as a fine loamy textured Dystric Fluvic Cambisol (Michéli et al., 2006) and have a mixed glacial till parent material.

Samples were taken from 4 replicates (different trees) of goat willow (*Salix caprea)*, alder (*Alnus* spp.) and oak (*Quercus* spp.) at sites 1-3 (only 1 replicate of willow at site 3 in September). Samples were taken from additional species: sycamore (*Acer pseudoplatanus*), common ash (*Fraxinus excelsior)*, european beech (*Fagus sylvatica*) and sweet chestnut (*Castanea sativa*) from trees forming part of Bangor Diverse forestry project site 3, with replicates of alder and sycamore also taken from the silvopastoral agroforestry site 4.

2.2 *Sample collection and processing*

Leaf samples were collected during June (Spring) and September prior to leaf senescence (early Autumn). To obtain the leaf samples, branches with a diameter of 10 mm were selected from four orientations (North, East, South, West) around the tree crown and cut using secateurs. Green biomass (~600 g) was collected by stripping the leaves and petioles from the cut branches using a gloved hand into paper bags and oven dried at 40 °C until constant mass. Dried leaf material was ground to pass a 2 mm screen prior to analysis.

2.3. Mineral analysis

For all elements measured, except iodine, dried samples were weighed (0.1-0.2 g) into a high pressure digestion vessel (HVT50, Anton Paar, St Albans, UK) and to this 3 ml 68% nitric acid (Primar plus, Fisher Scientific, Loughborough, UK), 3 ml deionised water (17 MΩ, Purite hp 160, Suez, Thame, UK.) and 2 ml 30% hydrogen peroxide (Analar, VWR Ltd, Lutterworth UK) was added before being run on a digest Microwave (multiwavepro, Anton Parr, St Albans, UK) with a 10 minute ramp to 140 °C, 20 minute hold at 140 °C and subsequent cooling to 55 °C. Digested contents were transferred to a 25 ml universal tube (Sarstedt, Leicester UK) with 7 ml deionised water (as above). Blanks and appropriate standards/certified reference material was included with each batch run. After dilution 1 in 20 (0.5 ml in 10 ml) with 0.5 % nitric acid (diluted from 68 % Primar plus, Fisher Scientific, Loughborough, UK) into 14 ml (105 mm x 16.8 mm) polypropylene tubes (Sarstedt, Leicester UK), multi-element analysis (Ca, P, Mg, Na, K, Cu, S, Fe, Mo, Mn, Pb, Cd, As, B, Al, Ni, Se, Co, Zn) was undertaken. This was via ICPMS (Thermo-Fisher iCAP-Q, Thermo-Fisher Scientific, Loughborough, UK) with a ‘Flatopole collision cell’ (charged with helium gas for all elements except selenium where it was charged with hydrogen – changes within sample) upstream of the analytical quadrupole to reduce polyatomic interferences. Internal standards were introduced to the sample stream via a T-piece and included Sc (50 μg L^{-1}), Ge (20 μg L^{-1}) Rh (10 μg L^{-1}) and Ir (5 μg L^{1}) in a matrix of 2 % HNO_3. External calibration standards were usually all in the range 0 – 100 μg L^{-1} (ppb) for trace elements and 0 – 100 mg L^{-1} (ppm) for macro elements. Samples were introduced via a covered autosampler (Cetac ASX-520) through a 1317090 pfa-st nebulizer (ESI) (Thermo-Fisher Scientific, Loughborough, UK). Sample processing was undertaken using 'Qtegra software' (Thermo-Fisher Scientific, Loughborough, UK).

For iodine, approximately 0.1-0.2 g of dry material was accurately weighed into a 50 ml centrifuge tube (Falcon, Fisher Scientific, Loughborough, UK) a 5% solution of TMAH prepared by diluting 25 % TMAH (tetramethylammonium hydroxide 25 % W/W VWR Ltd, Lutterworth, UK) with deionised water (17 MΩ, Purite hp 160, Suez, Thame, UK.). This was vortex mixed and incubated at 70 °C for 4 hours with additional vortex mixing after approximately 2 hours and at the end of the incubation. After the incubation volumes were made up to 25 ml to achieve a final TMAH concentration of 0.2 %. Samples were mixed and centrifuged at 2000 g for 10 mins (Allegra x22, Beckman Coulter, High Wycombe, UK) before decanting into 14 ml (105 mm × 16.8 mm) polypropylene tubes (Sarstedt, Leicester UK) for ICP analysis. Blanks and appropriate standards/certified reference material was included with each batch run. Iodine analysis was run on decanted extract using ICPMS (Thermo-Fisher iCAP-Q) with the internal standard (5 ppb Re in 1% TMAH) introduced to the sample stream via a T-piece. External calibration standards were usually all in the range 0 – 100 μg L^{-1} (ppb). Samples were introduced via a covered autosampler (Cetac ASX-520) through a 1317090 pfa-st nebulizer (ESI) (ThermoFisher Scientific, Loughborough, UK). Sample processing was undertaken using ‘Qtegra software’ (Thermo-Fisher Scientific, Loughborough, UK).

2.4. Energy, protein and fibre analysis

Crude protein (CP) was determined by Kjeldahl digestion and potentiometric titration using a Gerhardt Vapodest 50S (Gerhardt Analytical Systems, C Gerhardt GmbH & Co, Germany). Modified acid digestible fibre (MADF) was measured following the methods of Kitcherside et al. (2000) using a FibreCapTM 2021/2023 system (FOSS Analytical, Denmark). Metabolisable energy (ME) was determined by wet chemistry (SAC Commercial Ltd, Penicuik, UK).

2.5. Statistical analysis

Data was collated and figures produced with Microsoft Excel Version 15.0 (Microsoft Corporation, Redmond, WA, USA). Principal component analysis (PCA) was conducted using SPSS 25.0 for windows (SPSS Inc. Chicago, IL, USA) and the PCA figure prepared using SigmaPlot 13.0 (Systat Software Inc., Chicago, IL, USA). A general linear model (ANOVA) was used to determine statistical differences with factors of site, time and species followed by a multiple-comparison Bonferroni posthoc test with an alpha of 0.05 where appropriate (MINITAB 17.1, Coventry, UK).

3. Results

The results for the trace elements cobalt, selenium, zinc and iodine which are important within grazing systems are shown in figures 1, 2, 3 and 4 respectively. Part a in each shows the three trees common across all sites. Within this there is a significant effect of tree species on cobalt concentration ($P<0.001$), with willow significantly higher than oak and alder which did not differ. Tree species also was significant for zinc ($P<0.001$) with willow significantly higher ($P<0.05$) than alder which was significantly higher ($P<0.05$) than oak. Selenium and iodine had no tree species effect but both had a significant site effect ($P<0.001$) with site 1 significantly higher ($P<0.05$) than either other site which did not differ statistically for selenium and for iodine site 3 was significantly higher than site 2 ($P<0.05$) which was greater than site 1 ($P<0.05$). For cobalt there was also a site effect ($P<0.05$) with site 1 significantly higher than site 2 and site 3 not statistically different from either of the other two sites. Iodine had a season effect with the September sample significantly higher ($P<0.001$) than June. There were no other significant effects of site, season or tree species for these 4 elements.

Other mineral elements analysed are presented in the supplementary data. There were significant main effects for site at $P<0.001$ for Cu, Mn, Na; $P<0.01$ for Cd, K and $P<0.05$ for Fe, P, Pb, S. Season main effects were significant at $P<0.001$ for B, Ca, Cu, P, Pb and $P<0.01$ for Na. For tree species, main effects were significant at $P<0.001$ for B, Ca, Cd, Cu, K, Mo, S; $P<0.01$ for Mg, Mn and $P<0.05$ for Fe, P.

The results for ME and CP, are shown in figures 5 and 6. The main effect model showed a significant effect of tree species for ME ($P<0.001$) with alder significantly greater ($P<0.05$) than oak which was significantly greater ($P<0.05$) than willow.

There was no effect of season or site. CP was significantly higher in June ($P<0.001$). There was a lower significant effect of tree species ($P<0.05$) with alder significantly higher than oak ($P<0.05$) and neither significantly different to willow.

Principal Component Analysis (PCA) of the three main species oak, alder, willow with mineral, ME and CP data revealed that the first two principal components explained 21.0% and 17.9% of the variation within the data (Figure 7). The three tree species were clearly separated along both PC axis with PC1 providing the best degree of separation. In agreement with the general linear model analysis, Co, Zn, Cd and Mo were more closely associated with willow, whereas ME, and to a lesser extent CP, was associated with alder. PC2 was strongly correlated with seasonality, with the leaf mineral content of willow also being greatest in the autumn (September) whereas the spring (June) and oak showed the lowest utility of fodder for supplementary feed.

Analysis of all eight tree species and the association of sites with mineral content, CP and ME resulted in two principal components that explained 22.4 and 16.9% (Figure 8). Here, Zn, Cd, and Co were associated with willow whereas ME and CP were associated with the two species of alder. The other species were not well separated, with the exception of ash which was associated with Ca, Mg, S and to a lesser extent P.

4. Discussion

The analysis so far has been on each species of tree leaf alone. It is very unlikely in practice that grazing sheep would only have access to a single leaf species. It is much more likely that the leaves would form part of a composite grazing platform alongside grass, clover, herbal leys and even other tree species. However, to make sure we cover the worst case scenario for leaves from each species then we have to discuss feeding each leaf species alone.

If livestock were fed on a diet of leaves alone and not with grass, other grazing or a supplementary feed then they would all be able to support a decent growth rate of between 150 and 250g/day for 20-40 kg lambs (AHDB, 2014). Alder was the best tree species in terms of both ME and CP supply of those on the 3 sites. However, whilst the current results indicate that alder could be an ideal source of fodder both in terms of energy and protein, it is consistently one of the least palatable trees for livestock, scoring lowest in palatability in the Woodland Grazing Toolbox (Forestry Commission Scotland, 2016), and a species that is typically left unprotected when other tree species require guarding against deer in the same environment. The reason for its low attractiveness as a voluntary feed source is unclear.

Within the trace elements, selenium was clearly more affected by site than species, a result most likely related to the selenium content of the underlying topsoil (Rawlins et al., 2012). Apart from site 1 which just about reached the potential maximum selenium requirement, the other sites were around the minimum selenium requirement and would need additional selenium to support production required in a grazing system. Grassland at site 2 was also marginal in selenium (Kendall et al., 2017). Cobalt and zinc concentrations of leaves were much higher for willow than for any other tree species; this was clearly significant across all of the sites ($P<0.001$). Cobalt and zinc concentrations of willow leaves exceeded the maximum requirements approximately 6 to 10 fold. This raises the potential for the use of willow as a supplementation strategy to augment deficient grazing platforms. A willow leaf intake of <10 % dry matter would potentially supply all, or most, of the zinc and especially cobalt dietary requirements, allowing the grass/other sward components to provide the additional energy and protein for growth. This would more than make up for the slightly lower energy and protein of the willow leaves themselves. A mechanistic understanding of cobalt and zinc hyperaccumulation by willow than by other species could be a useful focus of grass breeders to improve the nutritional content of grazed sward (Balafrej et al., 2020). Willow has previously been used for decontamination of heavy metal contaminated land (Volk et al., 2006) due to the enhanced uptake and partitioning away from key metabolic areas (Harada et al, 2011). In contrast most of the other tree leaves failed to supply the minimum cobalt requirement and none were able to supply the higher requirement for lambs. The other tree species were between minimum and maximum requirements for zinc, with performance only likely to be compromised in the fastest growing sheep, except for oak and ash.

Other elements mentioned within the introduction as being elevated were willow for magnesium (Robinson et al., 2005). However, we found that magnesium was higher (data not shown, see supplementary data) for alder than willow (or oak) with ash having the highest concentration. All concentrations were in excess of the highest sheep requirement (NRC, 2007).

In addition to the nutritional value of their leaves, the suitability of a tree species for use as a source of fodder is also related to its productivity and response to browsing or fodder management. The species included in this study represent different life strategies characterising ontogenesis (reproduction and growth biology) and ecological responses (e.g. shade tolerance, frost resistance). Willow and alder are both classified as ruderal or pioneer species which respond well to disturbance and have a high growth rate during establishment (Brzeziecki et al, 1994). Willow, and to a lesser extent, alder, are both species used for bioenergy production through short rotation coppicing, due to rapid re-growth after harvesting, and these characteristics also make them suitable for management as fodder trees. A potential additional benefit of alder is that it fixes nitrogen through association with the Actinobacteria *Frankia alni* which forms nodules on the root systems. Fixation levels of N by grey alder have been measured in unfertilised stands as 30-185 kg N/ha/yr (Jørgensen et al., 2005). Compared to other broadleaved species, alder leaves have high N concentrations even at leaf fall in autumn, so can contribute to improving soil quality (and potentially pasture productivity).

By contrast, oak is classified as a competitive stress tolerator (Brzeziecki et al., 1994), with low initial growth rates but a higher level of resistance to stress due to welldeveloped root systems. While perhaps less suitable as a regular fodder source due to slow re-growth rates, they potentially have a role to play since oak is a species long associated with pasture.

There are many factors affecting intake of tree fodder in addition to nutritional value or presence of anti-herbivorous compounds such as tannins. These include, the presence and condition of alternative feed resources, accessibility of the browse, feed novelty and familiarity, health status of the animal, and palatability.

Palatability is often regarded as a measure of how pleasant a feed source is to eat. This is too simplistic however since palatability is influenced by a rather complex relationship between the hedonistic sensation of (primarily) taste and feedback from post-ingestive processes. Therefore, both the food itself and an animal's reaction to the food can influence what and how much is eaten at any given time (Kearney et al., 2016). For example, lambs deficient in vitamin E preferentially selected a less attractive feed source that was high in vitamin E until balance had been restored, after which they returned to feeding on a more attractive source of food (Amanoel et al., 2016). The authors further noted that the artificially induced deficiency caused a change in feeding behaviour before any physiological outcomes could be detected, suggesting a high level of sensitivity to deficiency already developed in juvenile sheep. Inappetence is also a clinical sign in both zinc and cobalt deficiencies and selective behaviours for these minerals have also been noted (Kendall and Telfer, 2000). Alder is the only species included with known palatability concerns (Forestry Commission Scotland, 2016).

An increase in selective feeding on plants with high levels of condensed tannins can similarly occur in animals with intestinal parasite burdens and studies of sheep and goats show that feeding on tannin rich browse can reduce faecal egg counts by half (Min and Hart, 2003). When ingested, condensed tannins have a direct anthelmintic effect on multiple species of parasites within the gastrointestinal tract so that fewer eggs hatch, fewer larvae mature to adults and those that do are smaller and therefore produce fewer eggs (Waller et al., 2001; Novobilský et al., 2011; Williams et al., 2014). Tannin levels and composition can both influence a plant's animal-health properties and future studies could focus on unravelling the seasonal variation in the tannin profiles of tree fodder and their relationship to animal health. As well as the tannin effect on gastrointestinal parasites, many other trace elements have also been shown to have a role, for example cobalt deficiency leads to an impaired immune function which is linked to an increased susceptibility to parasite burdens in grazing livestock (Vellema et al., 1996; Paterson and MacPherson, 1990). This link is included in the SCOPS manual for parasite management in sheep (Abbott et al. 2012) and both effects add roles for the use of browse in the reduction of reliance on anthelmintic drenches, and the increasing resistance issues they have. Further work is required to look at the combined anthelmintic properties, especially from willow with its high cobalt and zinc in addition to tannins.

In conclusion, leaves from the tree species tested were able to fulfil the majority of the protein and energy requirement of growing lambs, with alder the best species in this regard. In terms of mineral concentration, oak was generally the lowest often unable to fulfil requirements, whilst willow in the case of zinc and cobalt had concentrations of magnitudes above the requirement allowing for consideration of use as a biological supplement. Tree leaves should be suitable for use alongside grazing as an additional fodder source and if fed as the major dietary component (e.g. in times of drought) should not compromise potential lamb growth. Ruminant livestock farmers should consider increasing tree cover to secure health, welfare and performance benefits.

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Figure Headers

Figure 1 cobalt concentration (mg/kg DM) of tree leaves in June (dark grey) and September (lighter grey) from a) three tree species (oak, willow, alder) across the 3 main sites, and b) from sites 3 and 4 including additional tree species (sycamore, ash, beech and chestnut). The solid black line indicates the upper limit of the range of sheep requirements, with the dotted black line indicating the lower limit of the range of requirements.

Figure 2 selenium concentration (mg/kg DM) of tree leaves in June (dark grey) and September (lighter grey) from a) three tree species (oak, willow, alder) across the 3 main sites, and b) from sites 3 and 4 including additional tree species (sycamore, ash, beech and chestnut). The solid black line indicates the upper limit of the range of sheep requirements, with the dotted black line indicating the lower limit of the range of requirements.

Figure 3 zinc concentration (mg/kg DM) of tree leaves in June (dark grey) and September (lighter grey) from a) three tree species (oak, willow, alder) across the 3 main sites, and b) from sites 3 and 4 including additional tree species (sycamore, ash, beech and chestnut). The solid black line indicates the upper limit of the range of sheep requirements, with the dotted black line indicating the lower limit of the range of requirements.

Figure 4 iodine concentration (mg/kg DM) of tree leaves in June (dark grey) and September (lighter grey) from a) three tree species (oak, willow, alder) across the 3 main sites, and b) from sites 3 and 4 including additional tree species (sycamore, ash, beech and chestnut). The solid black line indicates the upper limit of the range of sheep requirements, with the dotted black line indicating the lower limit of the range of requirements.

Figure 5 metabolisable energy (ME) content (MJ/kg DM) of tree leaves in June (dark grey) and September (lighter grey) from a) three tree species (oak, willow, alder) across the 3 main sites, and b) from sites 3 and 4 including additional tree species (sycamore, ash, beech and chestnut). The solid black line indicates the requirement of a 20kg lamb growing at 250 g/day, with the dotted black line indicating the requirement of a 40kg lamb growing at 150 g/day (AHDB, 2014).

Figure 6 crude protein (CP) content (g/kg DM) of tree leaves in June (dark grey) and September (lighter grey) from a) three tree species (oak, willow, alder) across the 3 main sites, and b) from sites 3 and 4 including additional tree species (sycamore, ash, beech and chestnut). The solid black line indicates the requirement of a 20kg lamb growing at 250 g/day, with the dotted black line indicating the requirement of a 40kg lamb growing at 150 g/day (AHDB, 2014).

Figure 7. Principal component analysis of the tree species common alder, English oak and goat willow treatments using leaf litter mineral, crude protein and metabolisable energy data collected in the spring and autumn. Error bars represent ± 1 SE.

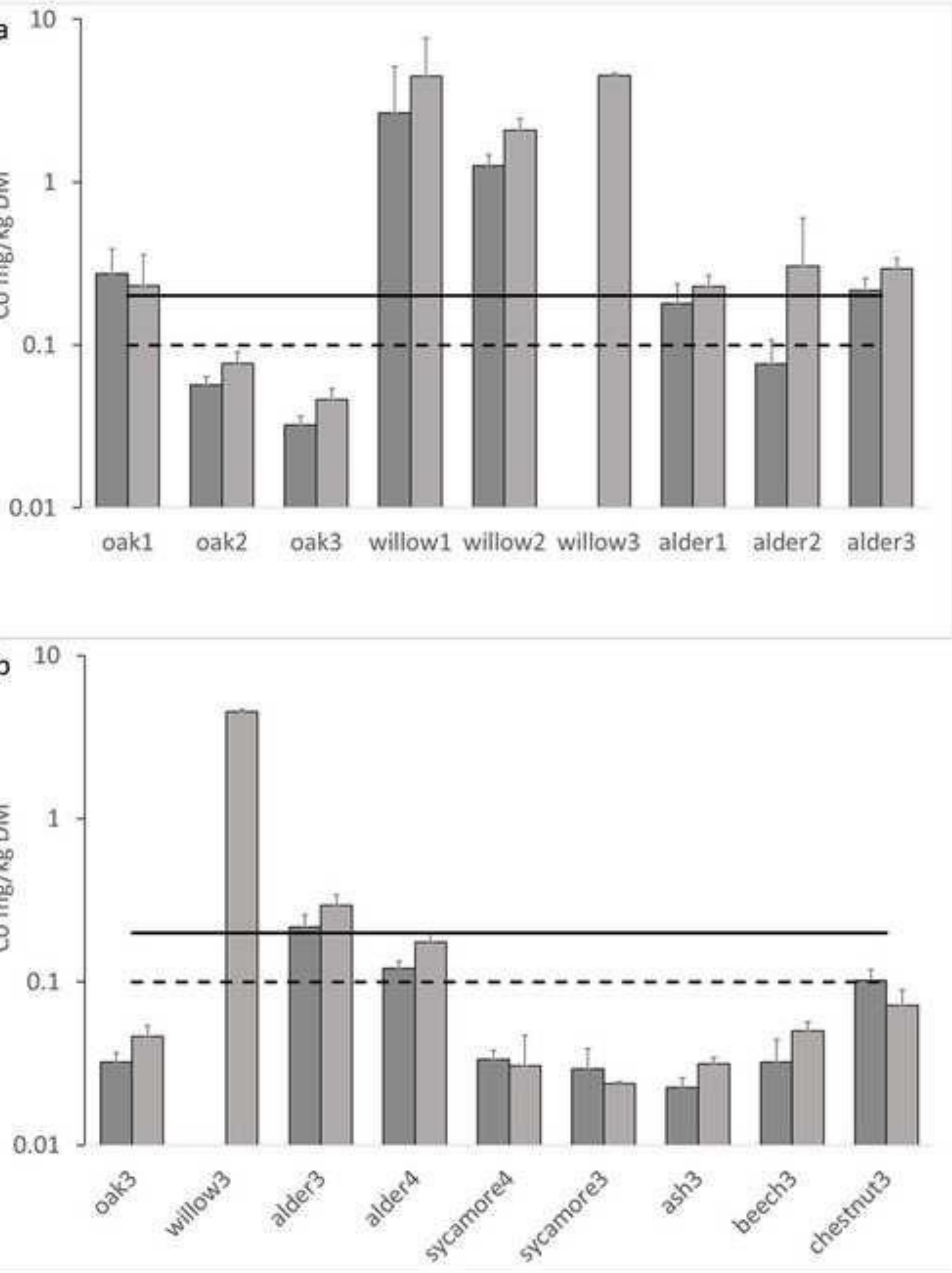
Figure 8 Principal component analysis of the tree species common alder, English oak goat willow, sweet chestnut, silver birch, European beech and Sycamore treatments using leaf litter mineral, crude protein and metabolisable energy data collected in the spring and autumn. Panel A shows the association of species and the three sites (1,2,3+4) and Panel B shows the leaf mineral, crude protein and metabolisable energy content. Error bars represent ± 1 SE.

		oak-1	oak-2	oak-3	willow-1	willow-2	willow-3	alder-1	alder-2	alder-3
Ca	june	8.6±1.29	8.2±1.44	8.7±1.65	10±3.01	9.5±0.55		14.8±1.2	9.3±1.65	11.5±1.71
g/kgDM	sept.	10.2±0.71	12.2±0.62	10.1±1.54	13.8±3.32	16.9±3.33	22.3±0.7	18±1.97	15.1±0.65	18.1±0.9
P	june	2.4±0.24	1.8±0.31	2.6±0.16	2.6±0.25	2.7±0.15		2.8±0.29	1.9±0.26	2.5±0.27
g/kgDM	sept.	1.8±0.46	1.5±0.11	2.6±0.48	1.8±0.22	2.3±0.58	1.4±0.05	1.8±0.11	1.5±0.15	2.2±0.03
Mg	june	2.3±0.84	1.7±0.55	2.8±0.23	1.8±0.19	2.1±0.33		3.1±0.15	2.1±0.34	2.9±0.22
g/kgDM	sept.	1.3±0.34	1.3±0.34	2.6±0.7	1.7±0.37	2.7±0.81	2.3±0.09	2.6±0.45	2.6±0.28	3.7±0.15
Na	june	0.16±0.078	0.28±0.08	0.2±0.074	0.07±0.026	0.23±0.037		0.16±0.03	0.15±0.046	0.15±0.03
g/kgDM	sept.	0.2±0.073	0.41±0.08	0.53±0.125	0.1±0.036	0.24±0.066	2.53±0.01	0.23±0.019	0.18±0.007	0.52±0.157
K	june	13.2±2.22	9.6±2.48	11.7±0.92	17.3±1.23	12±0.4		10.7±1.22	6.7±1.49	11.9±0.94
g/kgDM	sept.	13.3±1.73	12.6±1.78	12.2±0.66	14.3±1.97	15.4±2.88	7.9±0.42	9.6±0.86	7.3±1.48	11.2±2.15
S	june	1.7±0.19	1.3±0.74	1.9±0.06	2.4±0.22	1.4±0.39		2.3±0.15	0.9±0.14	2.3±0.25
g/kgDM	sept.	1.2±0.07	1.5±0.06	1.4±0.17	2.4±0.16	2.8±0.48	2.1±0.04	1.9±0.03	2±0.14	1.9±0.28
Cu	june	10.5±1.57	8.5±1.88	13.1±1.7	9.8±0.41	6.8±0.45		19.2±1.43	10±2.96	14.7±3.65
mg/kgDM	sept.	6.1±0.59	6.9±0.8	7.6±0.6	5.9±0.88	4.4±0.37	6.4±0.51	13.1±2.19	7.3±1.2	11.9±1.78
Fe	june	516±234.9	115±22.3	84±4.8	105±15.9	79±3.7		111±12.2	63±10	96±9.9
mg/kgDM	sept.	164±51.1	175±30.4	78±18.3	110±17.4	167±21.5	112±16	124±4.2	134±26	99±24
Mo	june	0.3±0.221	0.14±0.116	0.04±0.013	0.1±0.033	0.13±0.024		0.06±0.016	0.03±0.007	0.05±0.028
mg/kgDM	sept.	0.08±0.03	0.13±0.064	0.03±0.017	0.13±0.032	0.15±0.074	0.29±0.004	0.04±0.011	0.02±0.017	0.03±0.02
Mn	june	302±101.8	132±80.2	778±237.5	391±374.7	502±93		117±19.8	123±52.6	676±106.5
mg/kgDM	sept.	374±186.7	107±32.5	1171±222.9	668±520.9	949±228.8	208±7.5	70±16.6	283±305.3	858±105.3
Pb	june	0.27±0.094	0.29±0.089	0.13±0.016	0.23±0.072	0.26±0.085		0.31±0.061	0.24±0.102	0.22±0.033
mg/kgDM	sept.	0.43±0.159	0.45±0.072	0.2±0.031	0.34±0.066	0.57±0.099	0.8±0.046	0.41±0.078	0.48±0.084	0.27±0.026
Cd	june	0.05±0.025	0.03±0.019	0.05±0.015	1.72±0.726	5.3±1.736		0.01±0.002	0.02±0.011	0.02±0.004
mg/kgDM	sept.	0.03±0.011	0.03±0.011	0.04±0.01	2.58±1.55	6.25±2.224	1.41±0.048	0.01±0.002	0.01±0.001	0.02±0.007
As	june	0.22±0.121	0.03±0.009	0.02±0.004	0.04±0.02	0.02±0.008		0.03±0.005	0.02±0.009	0.04±0.003
mg/kgDM	sept.	0.06±0.025	0.06±0.009	0.03±0.006	0.05±0.013	0.07±0.008	0.17±0.002	0.04±0.004	0.05±0.013	0.04±0.003
B	june	29.4±2.14	29.3±5.49	33.7±5.16	19.2±4.43	16±1.71		46.9±7.24	27.4±4.73	26.5±1.31
mg/kgDM	sept.	43.4±4.02	55.5±13.46	41.2±17.37	31.4±11.57	32.6±9.69	42.9±0.93	44±8	40.8±5.85	25.7±4.44

Supplementary data table 1 mean (± s.d.) leaf mineral concentrations (g/kg DM for Ca, P, Mg, Na, K, S and mg/kg DM for Cu, Fe, Mo, Mn, Pb, Cd, As, B) for oak, willow and Alder trees across the 3 different sites (1,2,3).

		alder-4	sycamore-3	sycamore-4	ash-3	beech-3	chestnut-3
Ca	june	9.4±0.84	13.6±1.68	13.6±4.33	21±2.82	8.7±0.98	7.2±0.84
g/kgDM	sept.	15.9±1.64	21.9±1.2	23.5±4.25	30.3±0.83	10.8±0.62	10.1±1.57
P	june	2.4±0.19	2.5±0.24	2.7±0.29	3.1±0.41	1.8±0.09	2.1±0.14
g/kgDM	sept.	1.6±0.2	1.8±0.28	2.4±0.28	2.2±0.47	1.6±0.19	2.3±0.32
Mg	june	2±0.23	2.5±0.24	2±0.35	5.5±0.44	2±0.13	2.8±0.23
g/kgDM	sept.	2.3±0.25	3.1±0.17	2.4±0.5	6.7±1.07	2.4±0.09	3.2±0.39
Na	june	0.69±0.159	0.1±0.007	0.2±0.094	0.28±0.072	0.21±0.042	0.17±0.028
g/kgDM	sept.	1.41±0.472	0.44±0.107	0.99±0.135	0.93±0.259	0.69±0.138	0.67±0.156
K	june	15.1±2.45	17.2±0.55	21±0.94	15±3.49	8.7±0.68	10.2±0.65
g/kgDM	sept.	11.5±2.5	21.2±3.08	30.5±4.52	16±2.69	11.4±0.99	11.3±2.73
S	june	2.2±0.2	2.5±0.4	2.6±0.75	3.2±0.35	1.5±0.08	1.6±0.12
g/kgDM	sept.	1.8±0.11	1.7±0.16	1.8±0.11	3.3±0.53	1.3±0.09	1.3±0.03
Cu	june	12.3±1.9	9.2±0.6	7.5±0.62	11.4±1.82	9.4±0.81	11.1±0.28
mg/kgDM	sept.	8.2±1	5.2±1.08	5.5±1.11	7.1±0.57	7.1±0.91	5.4±0.16
Fe	june	110±7.4	88±9.2	91±10.4	79±5.6	84±6.2	110±12
mg/kgDM	sept.	118±33.3	87±6.8	93±16.7	78±15.2	72±1.2	98±21.2
Mo	june	0.04±0.027	0.03±0.012	0.04±0.027	0.26±0.032	0.05±0.025	0.06±0.024
mg/kgDM	sept.	0.04±0.039	0.03±0.016	0.04±0.023	0.18±0.084	0.03±0.006	0.05±0.018
Mn	june	490±167.7	253±63.2	435±211.3	73±6.6	820±473.2	632±91.9
mg/kgDM	sept.	737±154.3	683±746.7	808±64.3	99±19.4	962±260.5	938±128.6
Pb	june	0.22±0.008	0.17±0.024	0.17±0.074	0.24±0.193	0.16±0.026	0.22±0.028
mg/kgDM	sept.	0.34±0.09	0.22±0.042	0.18±0.051	0.21±0.04	0.23±0.019	0.22±0.053
Cd	june	0.02±0.004	0.18±0.027	0.26±0.074	0±0.001	0.08±0.031	0.08±0.015
mg/kgDM	sept.	0.01±0.002	0.29±0.178	0.36±0.053	0.01±0.004	0.07±0.015	0.07±0.006
As	june	0.05±0.007	0.03±0.006	0.04±0.012	0.03±0.004	0.02±0.006	0.04±0.005
mg/kgDM	sept.	0.05±0.012	0.04±0.005	0.06±0.024	0.05±0.011	0.03±0.003	0.04±0.007
B	june	33.3±4.38	31.7±4.74	31.9±10.7	37.9±7.54	29±3.65	30±2.14
mg/kgDM	sept.	26.1±5.1	35.1±4.04	30.3±5.48	31.4±2.8	24.3±3	29±3.4

Supplementary data table 2 mean (± s.d.) leaf mineral concentrations (g/kg DM for Ca, P, Mg, Na, K, S and mg/kg DM for Cu, Fe, Mo, Mn, Pb, Cd, As, B) for the additional trees at sites 3 and 4.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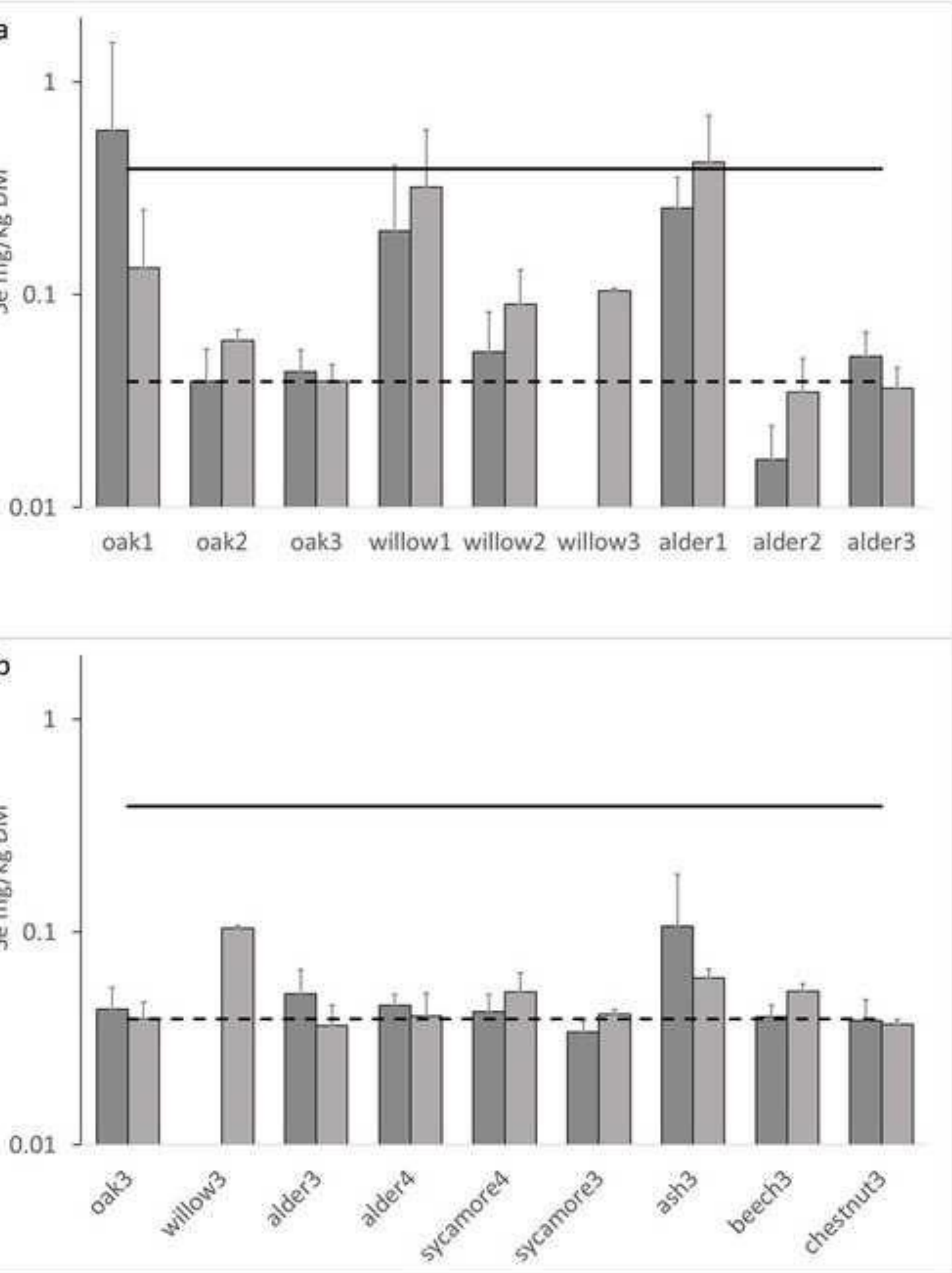
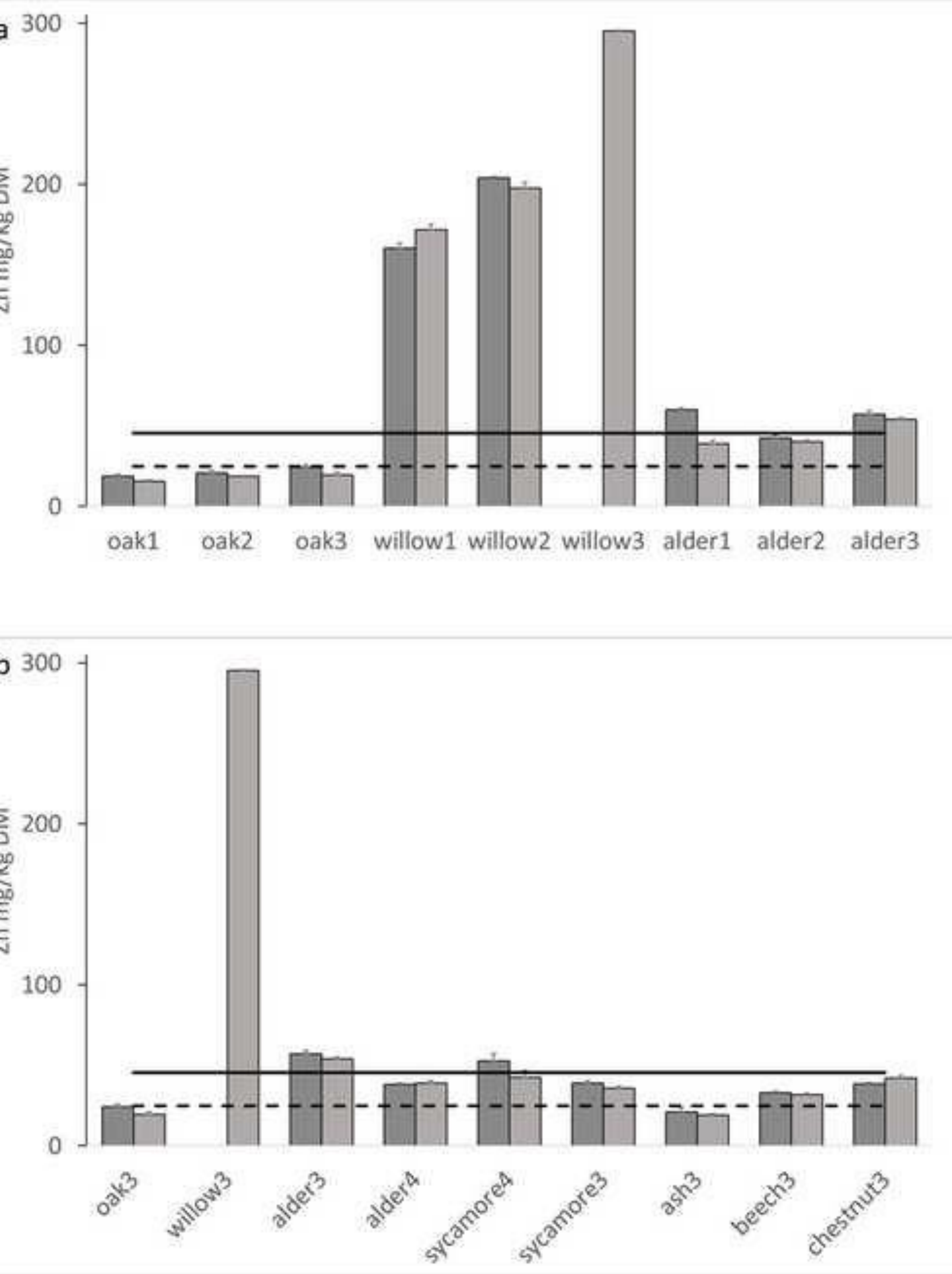
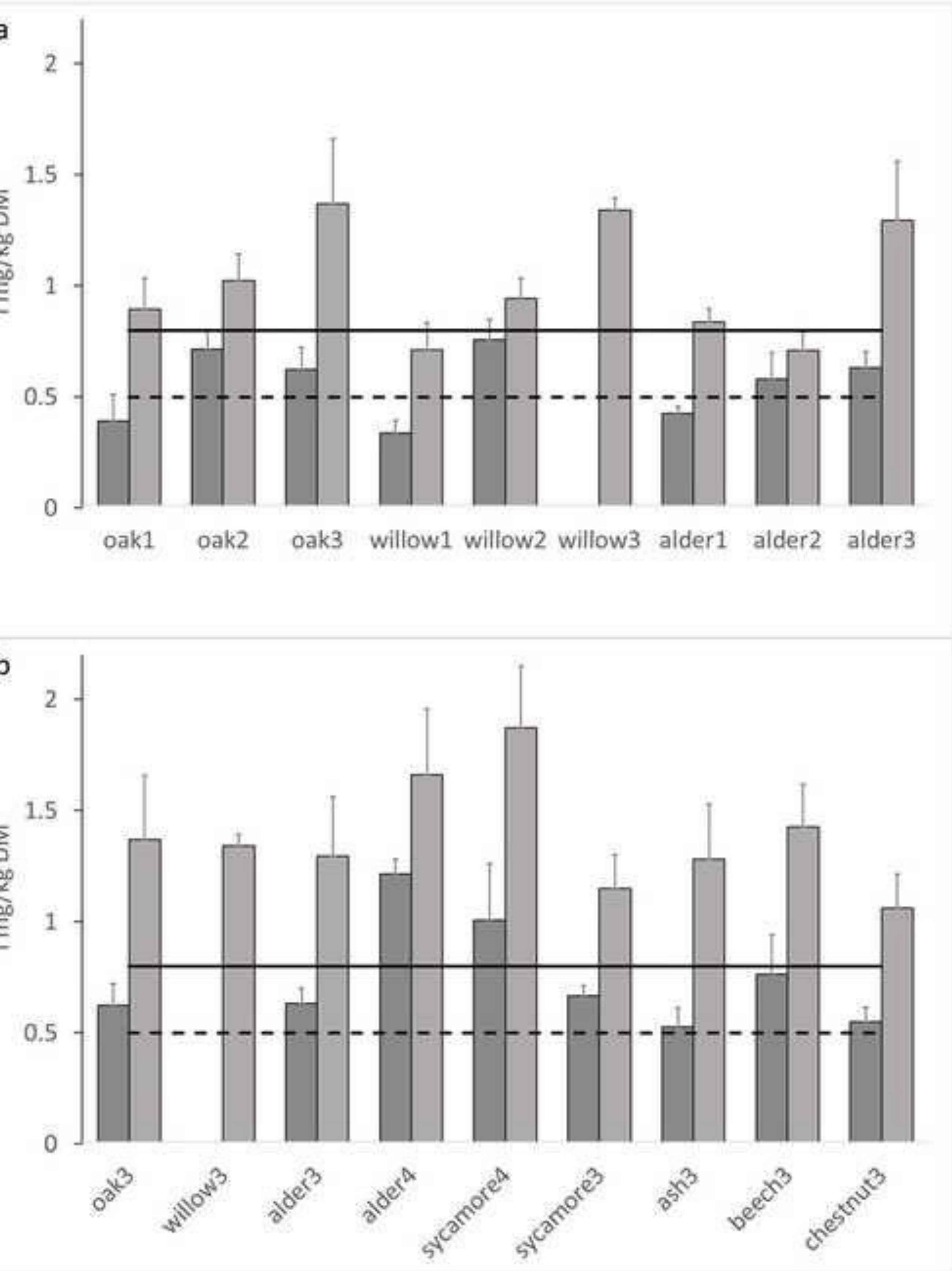
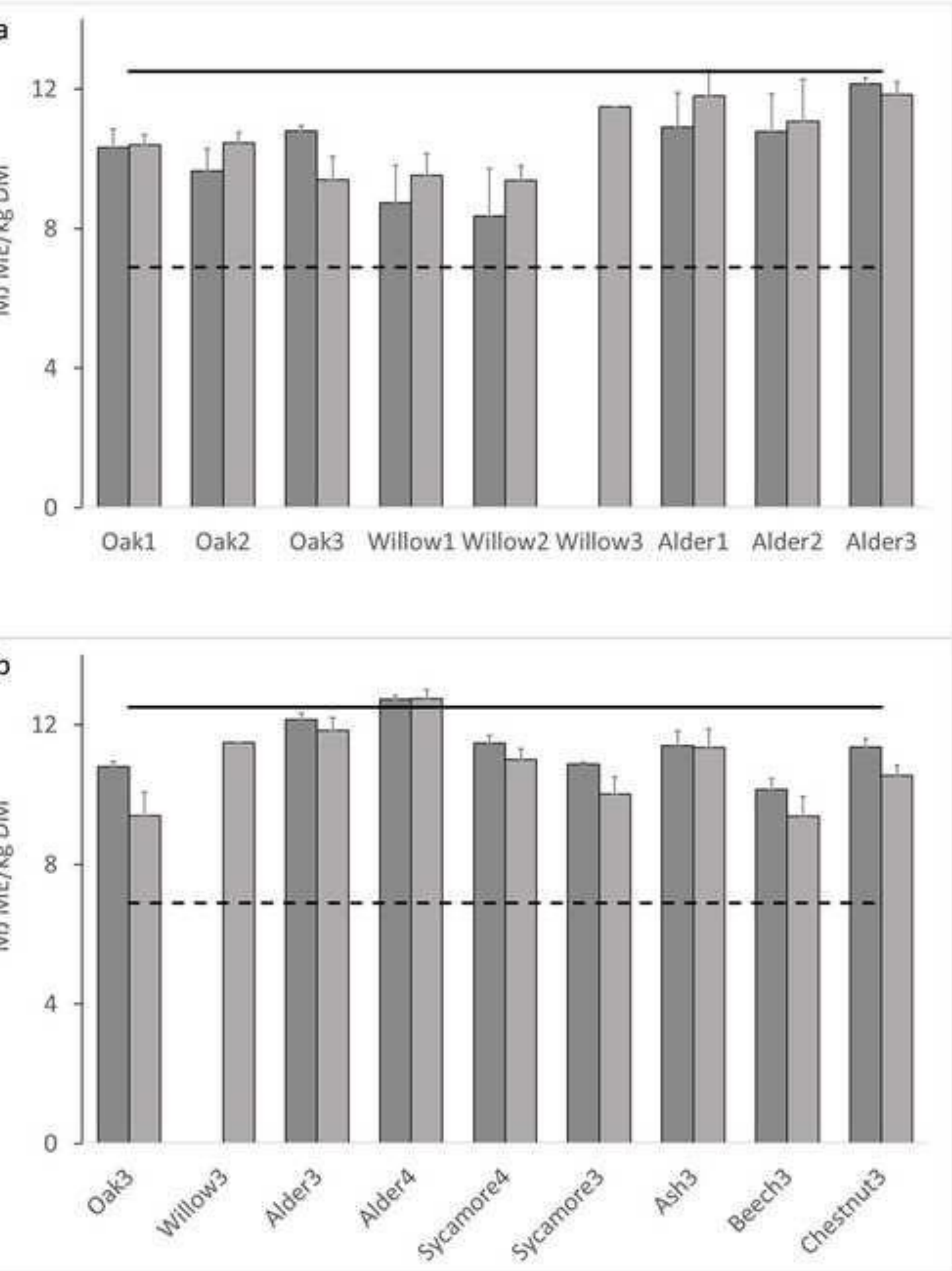
sycamore4
sycamore3
ash3
beech3
chestnut3

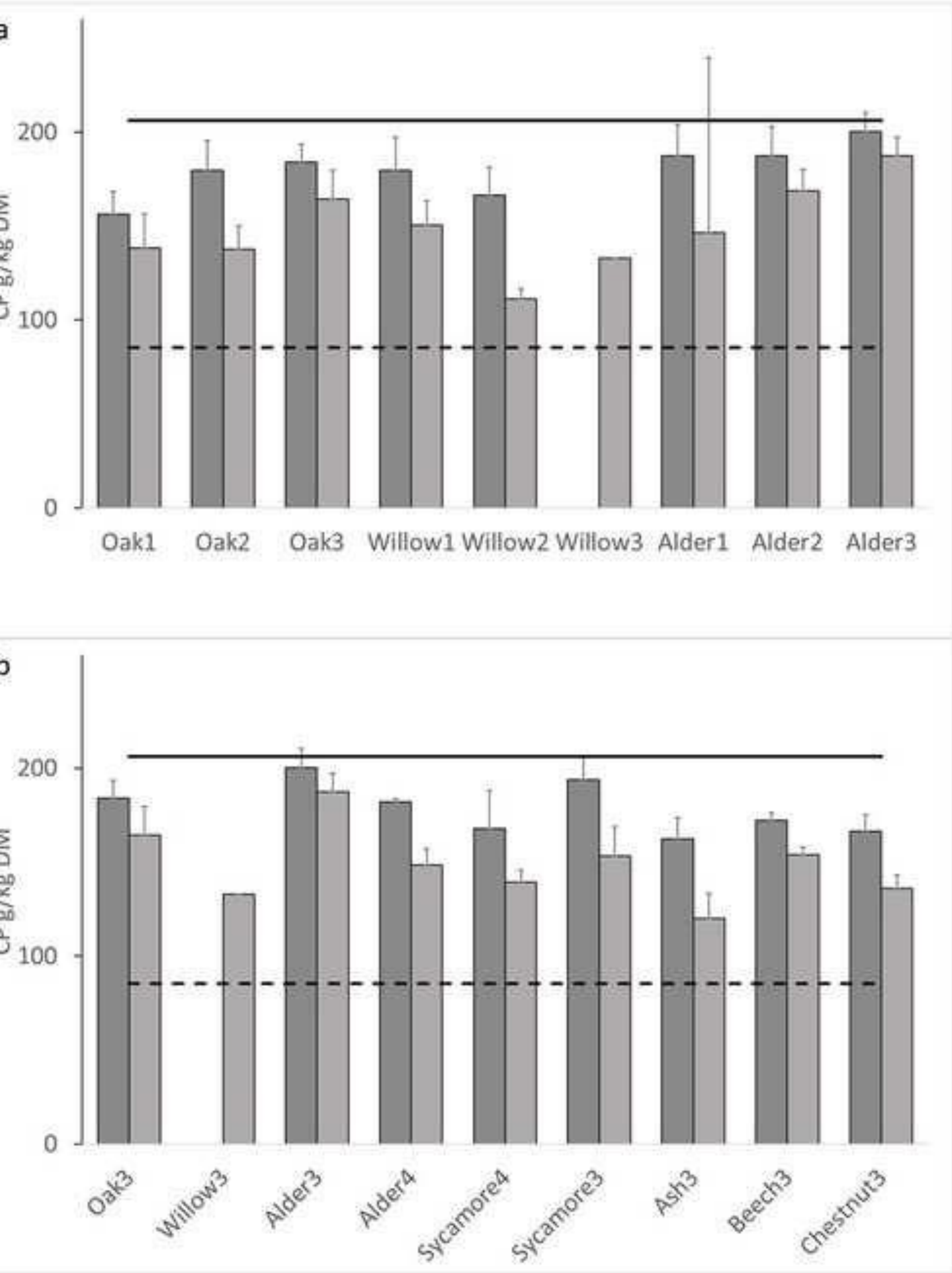

a
1
0.1
0.01
oak1
oak2
oak3
willow1
willow2
willow3
alder1
alder2
alder3
b
1
0.1
0.01
oak3
willow3
alder3
alder4
sycamore4
sycamore3
ash3
beech3
chestnut3

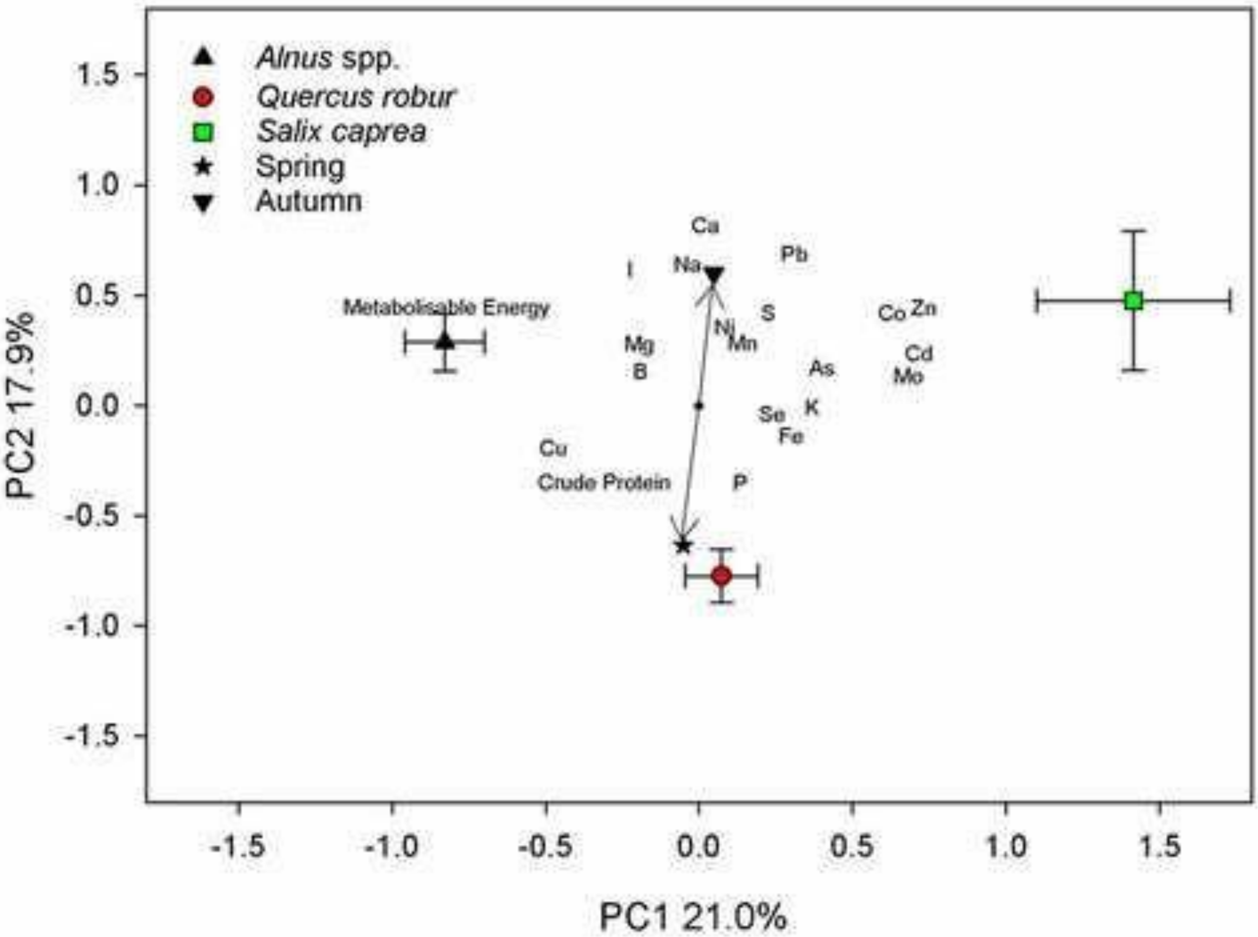

a
300
200
100
0
oak1
oak2
oak3
willow1
willow2
willow3
alder1
alder2
alder3
b
300
200
100
0
oak3
willow3
alder3
alder4
sycamore4
sycamore3
ash3
beech3
chestnut3


a
b
2
1.5
1
0.5
0
oak1
oak2
oak3
willow1
willow2
willow3
alder1
alder2
alder3
alder4
sycamore4
sycamore3
ash3
beech3
chestnut3

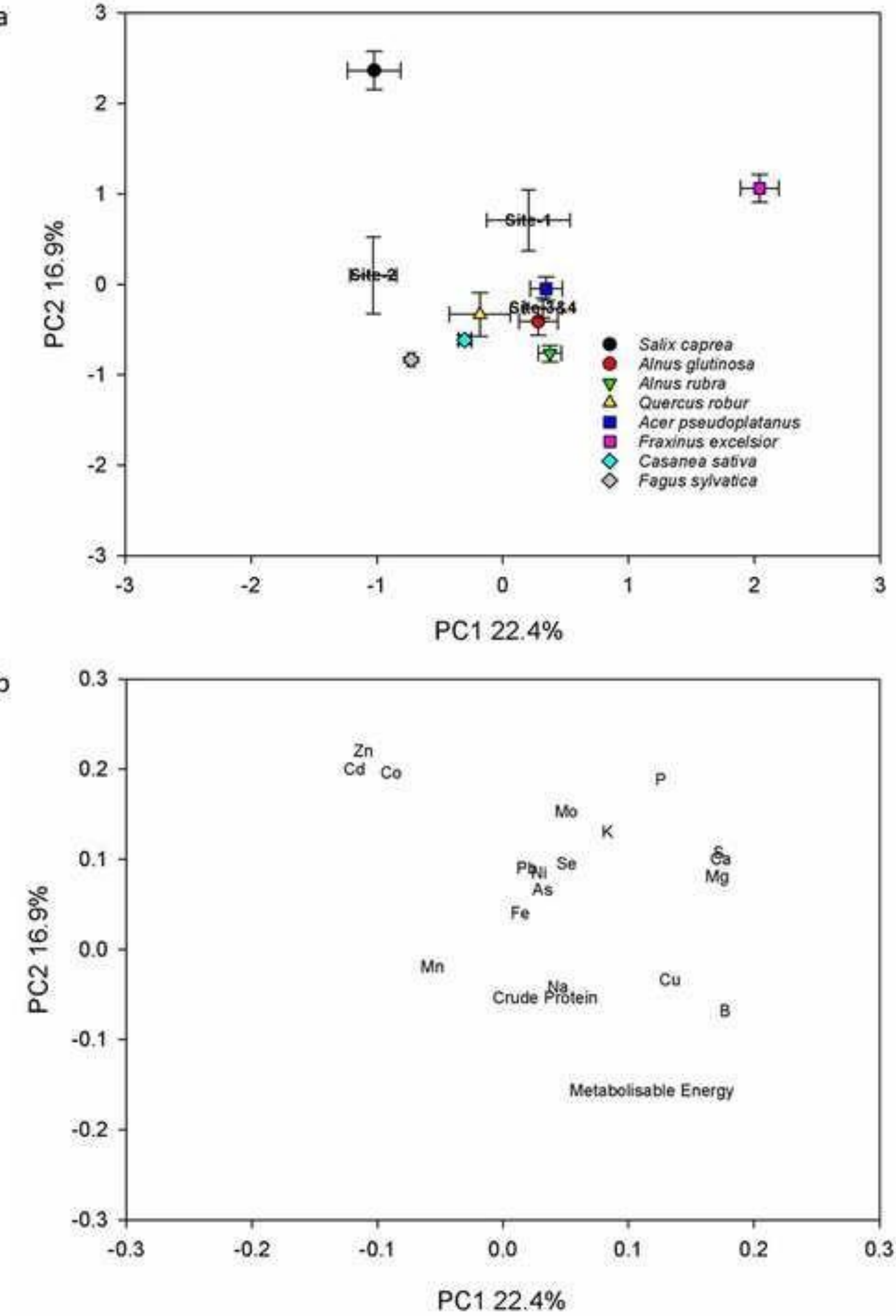


a
MJ ME/kg DM
0
4
8
12
Oak1
Oak2
Oak3
Willow1
Willow2
Willow3
Alder1
Alder2
Alder3
b
MJ ME/kg DM
0
4
8
12
Oak3
Willow3
Alder3
Alder4
Sycamore4
Sycamore3
Ash3
Beech3
Chestnut3


a
CP g/kg DM
200
100
0
Oak1
Oak2
Oak3
Willow1
Willow2
Willow3
Alder1
Alder2
Alder3
b
CP g/kg DM
200
100
0
Oak3
Willow3
Alder3
Alder4
Sycamore4
Sycamore3
Ash3
Beech3
Chestnut3



Alnus spp.
Quercus robur
Salix caprea
Spring
Autumn
PC2 17.9%
PC1 21.0%
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
Metabolisable Energy
Ca
Na
I
Pb
S
Co
Zn
Ni
Mn
Mg
B
As
Cd
Mo
Se
K
Fe
Cu
Crude Protein
P


a
Site-1
Site-2
Site-3&4
Salix caprea
Alnus glutinosa
Alnus rubra
Quercus robur
Acer pseudoplatanus
Fraxinus excelsior
Casanea sativa
Fagus sylvatica
PC2 16.9%
PC1 22.4%
b
Zn
Cd
Co
P
Mo
K
S
Ca
Mg
Pb
Ni
Se
As
Fe
Mn
Cu
Na
Crude Protein
B
Metabolisable Energy
PC2 16.9%
PC1 22.4%