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1 **Does geographic origin dictate ecological strategies in *Acacia senegal* (L.) Willd.?**

2 **Evidence from carbon and nitrogen stable isotopes**

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2

1 **Abstract**

2 *Background and Aims:* *Acacia senegal*, a leguminous dryland tree, is economically and  
3 ecologically important to sub-Saharan Africa. Water-use efficiency (WUE) and biological  
4 nitrogen fixation (BNF) are fundamental to plant productivity and survival. We quantify  
5 provenance differences in WUE, BNF, photosynthesis, biomass and gum arabic production  
6 from *A. senegal* assessing genetic improvement potential.

7 *Methods:* Using stable isotope ratios, we determined WUE ( $\delta^{13}\text{C}$ ) and BNF ( $\delta^{15}\text{N}$ ) from  
8 provenances of mature *A. senegal* in field-trials (Senegal), sampling leaves at the beginning  
9 (wet) and end (dry) of the rainy season. Seedling provenance trials (UK) determined  
10 photosynthesis, and biomass and  $\delta^{13}\text{C}$  in relation to water table. Environmental data were  
11 characterised for all provenances at their sites of origin.

12 *Results:* Provenances differed in both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Gum yield declined with increasing  
13 WUE. Virtually no BNF was detected during the dry season and seedlings and mature trees  
14 may have different WUE strategies. Wind speed and soil characteristics at provenance origin  
15 were correlated with isotope composition and gum production.

16 *Conclusion:* Provenance differences suggest that selection for desirable traits, e.g. increased  
17 gum production, may be possible. As ecological strategies relate to native locality, the  
18 environmental conditions at plantation site and provenance origin are important in assessing  
19 selection criteria.

20

1 **Key words**

2 Water use efficiency; biological nitrogen fixation; sub-Saharan Africa; stable isotopes

3 photosynthesis; light response curves; provenance

## 1 **Introduction**

2 Water and nitrogen availability are major determinants of plant production in natural, semi-  
3 natural and agricultural ecosystems (Bacon 2004; Sprent 1987; Unkovich et al. 2008; Werner  
4 and Newton 2005). To a large extent, the structure of plant populations, communities, and  
5 phenology is determined by the spatial and temporal distributions and availability of water  
6 and nitrogen (N) in the soil (Bacon 2004; Do et al. 2005; Werner and Newton 2005). Both  
7 biological nitrogen fixation (BNF) and water-use efficiency (WUE; the ratio of  
8 photosynthetic production to the rate of transpiration) can be intrinsic physiological  
9 characteristics for species and there is also evidence for intra-specific variation in both  
10 parameters (Bacon 2004; Werner and Newton 2005). It is likely that trade-offs exist between  
11 the benefits and costs of, for example, BNF and WUE;) in relation to plant productivity and  
12 survival. This may be particularly evident in dryland systems where the temporal and spatial  
13 variability of N and water vary greatly. N availability can enhance biomass production, and  
14 selection for WUE may have practical implications in dryland regions where there are  
15 constraints on productivity (Ripullone et al. 2004). Hence, the detection of genotypes that  
16 maintain plant productivity and optimise WUE and BNF in different environmental  
17 conditions is an important (though particularly challenging) goal especially in developing  
18 countries (Bacon 2004; Werner and Newton 2005) where livelihood is closely coupled to  
19 ecological benefit.

20 Soil water availability is a pre-requisite for BNF to occur, and is particularly crucial in the  
21 drylands where soil type and climate largely determine the temporal and spatial variability of  
22 soil water, and thus the occurrence of BNF. As such, the temporal and spatial variability of  
23 BNF is not always clear and it also may not always be the dominant process by which plants  
24 obtain N; plants are equally likely to source N from different N cycling process, e.g.

1 recycling of nitrogen from deeper layers in interstitial waters or from decomposing tissues  
2 (Dupuy and Dreyfus 1992; Sprent 1987).

3 In dryland ecosystems, increased WUE is a potentially valuable trait which might be  
4 expected to be under strong selection pressure and hence highly heritable. Though more than  
5 one strategy may be evident, plants might be perpetually conservative in water use, or have a  
6 high water use and/or productivity in the wet periods before water stress conditions return.

7 These strategies will depend on environmental factors and their temporal and spatial  
8 variability. Nevertheless, the potential for tree improvement would also be correspondingly  
9 high if improved genotypic/phenotypic WUE traits could be identified in species and  
10 associated with BNF. This would represent an ideal case where there is the potential not only  
11 to increase productivity but also to improve sustainability of land-use systems. Quantitative  
12 trait loci (QTL) analysis and genetic engineering are beginning to disentangle the genetic  
13 complexity surrounding adaptation to environmental conditions in crops (e.g. wheats), where  
14 it has been possible to enhance biomass production although this is mainly through improved  
15 soil water extraction efficiency than WUE *per se* (Tuberosa 2004). In *Quercus* spp.

16 differences of  $\Delta^{13}\text{C}$ , (in bulk leaf matter, wood and cellulose) and intrinsic water use relate  
17 more readily to differences in stomatal conductance and stomatal density than photosynthetic  
18 efficiency (Roussel et al. 2009). Different WUE strategies exist between plants with different  
19 photosynthetic pathways (C3; C4; CAM) but also there is increasing evidence that they exist  
20 between and within species (Deans and Munro 2004; Gebrekirstos et al. 2011; Hausmann et  
21 al. 2005). These differences can also be broadly maintained irrespective of season (Deans and  
22 Munro 2004). Thus, the identification of optimal genotypes particularly when they are native  
23 legumes is a priority for dryland areas in Africa. Sprent et al. (2010) suggest that with modern  
24 techniques for improving both plant and rhizobial germplasms, better exploitation of native  
25 legumes is an achievable goal in Africa.

1 *Acacia senegal* (L.) Willd., is arguably one of the most important sub-Saharan African trees,  
2 inhabiting savannah systems that are under threat of anthropogenic and climate-mediated  
3 degradation and that have seen substantial losses of habitat (Hejcmanova et al. 2010; Obeid  
4 and Seifeldi 1970). The resolution of the taxonomic debate surrounding the delimitation of a  
5 type for *Acacia* has seen the re-naming of the African *Acacia*'s and *A. senegal* to *Senegalia*  
6 *senegal* (L.) Britton. However, we have retained the use of *A. senegal* here for continuity  
7 with previous literature but more importantly because of the widespread familiarity of the  
8 African people with the name *Acacia* rather than *Senegalia*. *A. senegal* is a multi-purpose  
9 tree, producing 'gum arabic' (an economic staple in sub-Saharan regions) and used for animal  
10 fodder, multiple timber products, intercropping, firewood, food and medicines (see Fadl and  
11 El Sheikh 2010; Fagg and Allison 2004; Sprent et al. 2010). The collection and sale of gum  
12 arabic can be especially important as it is produced in the dry season when other income is  
13 limited (Fagg and Allison 2004) and it is possible for families to earn up to \$150 per month  
14 during this season. Phylogeographic studies suggest that *A. senegal* var. *senegal* has  
15 undergone some past fragmentation in its West African range offering the potential for  
16 provenances to have built up some adaptive differentiation (Odee et al. 2012). This may have  
17 arisen during parallel cycles of expansion and contraction of the lowland rainforest in  
18 response to climate change during earlier epochs (Maley 2001; Plana 2004). As a legume, it  
19 is important for BNF and is associated with nodulating rhizobial bacteria from mostly fast-  
20 growing genera *Rhizobium* and *Sinorhizobium* (Fall et al. 2008; Nick 1998; Odee et al. 2002).  
21 The improved nutrient status in the topsoil beneath trees that associate with nitrogen fixing  
22 bacteria is well documented (Näsholm et al. 2009; Sprent et al. 2010; Werner and Newton  
23 2005). Although some research is beginning to point to the existence of variation between  
24 provenance genotypes within *A. senegal* (see e.g. Raddad and Luukkanen 2006), the goal of

1 identifying optimal ecotypes or genotypes for any given environment requires much further  
2 research.

3 Differences in the ratios of stable isotopes in plant foliage are often used as surrogates for the  
4 quantification of BNF [ $^{15}\text{N}/^{14}\text{N}$ , abbreviated as  $\delta^{15}\text{N}$ ] and to reflect overall plant water use  
5 [ $^{13}\text{C}/^{12}\text{C}$ , abbreviated as  $\delta^{13}\text{C}$ ] (Farquhar et al. 1989). Here we present data on N and C  
6 isotope ratios for *A. senegal* to build on the current evidence base (Fagg and Allison 2004;  
7 Raddad et al. 2005; Raddad and Luukkanen 2006) and provide insights into existence of  
8 ecotypes (a genetically distinct geographic variety) within *A. senegal* at the provenance level.  
9 We examine WUE ( $\delta^{13}\text{C}$ ), BNF ( $\delta^{15}\text{N}$ ) in provenance trials in Senegal and glasshouse trials  
10 to quantify:

11 1. between provenance variation in :

- 12 a. WUE using leaf level  $\delta^{13}\text{C}$  as a surrogate for WUE;
- 13 b. Estimates of BNF using leaf levels of  $\delta^{15}\text{N}$ ;
- 14 c. biomass allocation and photosynthetic characteristics.

15 2. within provenance variation in:

- 16 a. WUE using leaf level  $\delta^{13}\text{C}$ ;

17 and

18 3. relate variation in WUE and BNF to the environmental variables of the ‘native’  
19 range of each provenance.

20



## 1 **Materials and Methods**

### 2 *Senegal Trials*

3 We used two provenance trials of *A. senegal* var. *senegal* (containing the same provenances  
4 from across much of the natural range of the species, but grown under different  
5 environmental conditions) that were set up in 1994 by Institut Sénégalais de Recherches  
6 Agricoles (ISRA) and Centre National de Recherche Forestières (CNRF) at Dahra and  
7 Bambey, Senegal (Figure 1 and Table 1). At both sites, a randomized block design of four  
8 blocks was used, within which 17 randomly assigned provenance plots were established, each  
9 containing twenty five randomly chosen plants from that provenance, planted at 5 m spacing.  
10 Note that a subset of 10 these provenance was used for sampling (see below) Bambey has  
11 soils typically composed of leached tropical ferruginous sandy clays, with a mean rainfall of  
12 around 400 mm yr<sup>-1</sup> and a rainy season extending from June to September. Dahra's dry  
13 *Acacia* woodlands are typically open to grazing, and on leached, tropical ferruginous soils  
14 (soil chemistry is detailed in Ndoye et al. 2012) ; it has a mean rainfall of around 250 mm yr<sup>-1</sup> and  
15 a slightly shorter rainy season from July to September. We concentrated most of our  
16 sampling on Dahra which is within the natural range for gum production, whereas wetter  
17 Bambey is not. Seeds for these trials had been collected using standard protocols (see e.g.  
18 FAO 1993) and trees were not inoculated prior to planting out at the sites. Tree height data is  
19 given in Table S1 in the supplementary material.

### 20 *Selection of Provenances*

21 Provenance trials have been used in forestry since the early 19th century for detecting  
22 populations with desirable characteristics for tree-breeding programmes (Guries 1990). The  
23 benefit of these 'common-garden' type studies, is that environmental conditions are  
24 controlled, and quantitative traits easily scored (Johnson et al. 2004). Despite the fact that

1 most trees are long lived, even seedling studies are justifiable since selection in trees is  
2 thought to operate most efficiently at very early developmental stages (Persson and Ståhl 1990;  
3 Petit and Hampe 2006). Thus such trials are the ideal experimental design for the detection of  
4 differences across provenances and are extremely important for studies of adaptation to  
5 climate change (Aitken et al. 2008; Kawecki and Ebert 2004; Mátyás 1996). For species such  
6 as *Acacia senegal* that is highly likely to show a wide plastic response, provenance trials  
7 allow the partitioning of variation from different sources. Here, we take plasticity to be  
8 defined as the capacity of a single genotype (provenance) to exhibit variable phenotypes in  
9 different environments (*sensu* Bradshaw 1965) and, so variation in the phenotype may be  
10 expressed as:

$$11 \quad VP = VG + VE + VG * E + Verror$$

12 Where:  $VP$  = Total phenotypic variance for a trait;

13  $VG$  = Genetic variance (proportion of phenotypic variation attributable to genes);

14  $VE$  = Environmental variance (proportion of variation caused by the environment);

15  $VG * E$  = Genotype x Environment interaction (Genetic variation for phenotypic  
16 plasticity);

17  $Verror$  = Unexplained variance, including developmental noise, measurement error,  
18 etc.

19 When the plants are grown in common environment trials  $VE$  and  $VG * E$  are minimised thus  
20 the remaining phenotypic variation is largely due to  $VG$ . Whilst there is undoubtedly a degree  
21 of plasticity, provenance level differences were assessed on the residual variation after the  
22 site, season and block were removed. Thus we can assume any provenance level detected is  
23 genetically controlled (see analysis below).

24 Selection of provenances for this study was based on gum yield data from both sites (for 2001  
25 and 2007-9). From all surviving trees at each time period we calculated a mean for each  
26 provenance and an abundance weighted mean. These were weighted according to the

1 proportion of trees in each provenance contributing to the gum production rather than all  
2 surviving trees, i.e. where there are few trees contributing to the production values this down-  
3 weights the resulting mean. However, both of these mean values were highly correlated  
4 (Pearson's  $r = 0.94$ ) and gave the same qualitative results. On the basis of this data, we  
5 selected ten provenances, ranked from low to high yielding (Table 1 and Figure 1).

### 6 *Sampling for Stable Isotope Studies*

7 We targeted our sampling towards the beginning (wet) and end (dry) of the rainy season in  
8 August and October 2009. Note that although we use the terminology 'dry season' here  
9 October is not really the dry season *per se* but the beginning of the dry season. This was for  
10 two reasons: firstly, we could not sample in the dry season since *Acacia senegal* sheds most  
11 of its leaves during this period: secondly, gum tapping is traditionally timed to occur when  
12 around half the leaves have fallen from the canopy towards the end of the rainy  
13 season/beginning of the dry and hence represents a crucial time period in the interpretation of  
14 our results. Site level differences in the August data can be examined in terms of differences  
15 in soil water since Bambey is a much wetter site and is located outside the zone of gum  
16 production and therefore allows a similar dry/wet comparison to the seasonal change.

17 At each site, leaf material was collected from the ten *A. senegal* provenances in each of four  
18 experimental blocks, in addition, reference plant material was also collected (see below); in  
19 total 200 samples were collected. For the August sampling campaign (wet season) all chosen  
20 provenances at both sites were included. In October 2009 (dry season) only the drier Dahra  
21 trial was sampled using a subset of the provenances analysed in the August campaign,  
22 namely Kordofan, Kankoussa, Kirane, Diaménar, Karofane and Ngane. At each harvest, five  
23 trees from each of the selected provenances in each block were randomly chosen from which  
24 mature fully expanded sun leaves were collected; the same trees were harvested during both

1 wet and dry sampling periods. Field samples were collected into porous paper bags to allow  
2 drying while preventing loss of pinnules. Thirty leaves for  $\delta^{13}\text{C}$  and 20 leaves for  $\delta^{15}\text{N}$  were  
3 bulked for isotope analysis for each tree. The dry season sample for one Kankoussa tree was  
4 lost and so this tree was eliminated from the analysis making 19 trees available for analysis.  
5 Additionally, from the Kankoussa provenance ( $\delta^{13}\text{C}$  only), again 5 trees were selected per  
6 block but 5 (wet season) and 10 (dry season) individual leaves were harvested from each tree  
7 and analysed separately to give some measure of within tree  $\delta^{13}\text{C}$  variability. The height of  
8 all the sampled trees was estimated using a Haglöf (Långsele, Sweden) HEC electronic  
9 clinometer.

10  $\delta^{13}\text{C}$  is negatively associated with water use efficiency over the period of dry mass  
11 production and under drought stress it is a good predictor of stomatal conductance at least for  
12 crop species (Condon et al. 2004). Thus, we interpret low  $\delta^{13}\text{C}$  values as indicative of high  
13 WUE and high  $\delta^{13}\text{C}$  values as indicative of low WUE.

14 In order to estimate BNF,  $\delta^{15}\text{N}$  was also analysed from leaves of non- $\text{N}_2$ -fixing reference  
15 species (i.e. from species that have no known BNF capability) occurring within the vicinity of  
16 the trial sites. As these species were not explicitly part of the experimental trials, sampling  
17 was on the edges of blocks and as would be expected, herbaceous plants were only available  
18 in the early wet season. Consequently, the non- $\text{N}_2$ -fixing reference plants were variably  
19 sampled depending on availability. They were identified and grouped as follows: trees,  
20 shrubs, herbs and grasses.

21 The  $^{15}\text{N}$  natural abundance method estimates nitrogen derived from the atmosphere (Nd<sub>fa</sub>) as  
22 the contribution of nitrogen in putative  $\text{N}_2$ -fixing legumes which originates from BNF,  
23 calculated according to Shearer & Kohl (1986) using the following equation:

24 
$$\% \text{Nd}_{\text{fa}} = (\delta^{15}\text{N}_{\text{ref}} - \delta^{15}\text{N}_{\text{leg}}) / (\delta^{15}\text{N}_{\text{ref}} - \delta^{15}\text{N}_{\text{fix}}) 100;$$

1 (where  $\delta^{15}\text{N}_{\text{ref}}$  is  $\delta^{15}\text{N}$  of non-fixing reference plants;  $\delta^{15}\text{N}_{\text{leg}}$  is  $\delta^{15}\text{N}$  of the putative  $\text{N}_2$ -fixing  
2 plant;  $\delta^{15}\text{N}_{\text{fix}}$  is  $\delta^{15}\text{N}$  of fixed  $\text{N}_2$ ). For  $\delta^{15}\text{N}_{\text{fix}}$ , a value of -2.52 ‰ of *A. senegal* seedlings  
3 grown in N-free environment and solely dependent on BNF was used as reported by Isaac et  
4 al. (2011). Of all the non- $\text{N}_2$ -fixing reference plant species, only *Balanites aegyptiaca* (L.)  
5 Del. had higher mean  $\delta^{15}\text{N}$  than *A. senegal* for samples collected during the wet season. Thus,  
6 BNF was estimated during the wet season using *B. aegyptiaca* as non- $\text{N}_2$ -fixing reference  
7 ( $\delta^{15}\text{N}_{\text{ref}}$ ) plant. The species has previously been used as a reference plant in estimating BNF  
8 in natural populations of *A. senegal* (Isaac et al. 2011). *B. aegyptiaca* is also a tree, which  
9 remains green all year and will have a more similar root architecture to *A. senegal* than  
10 herbaceous species.

11 For stable isotope analysis, a portable microwave oven (c.f. Arndt and Wanek 2002; Popp et  
12 al. 1996) was used to rapidly dry the leaves (Wavebox portable microwave, Model No. WBP-  
13 TP-660, output 660 w). We conducted a pilot test comparing conventional oven drying and  
14 the microwave technique using *Rumex acetosa* L. leaves. These leaves were split along the  
15 mid-rib, with one half oven dried and the other microwave dried. There was no difference in  
16  $\delta^{13}\text{C}$  values between oven and microwave dried leaves when instrumental error was taken  
17 into account (supplementary material, Figure S2). After drying, leaves were stored in paper  
18 bags inside air-tight plastic bags or boxes with self-indicating silica gel for transport.

### 19 *Glasshouse Trials*

20 Two common environment trials were established in a semi-controlled glasshouse  
21 environment in the UK, (conducted over the UK summer period June –August) set up to  
22 mimic tropical conditions (mean temperature 25 °C, maximum 35 °C, minimum 18 °C; mean  
23 relative humidity 65% maximum 95% and minimum 30%). We used seeds of several  
24 provenances sourced from the World Agroforestry Centre (ICRAF) and wild collections (Fig

1 1a and Table 1). These covered the entire ecological range represented by the provenances  
2 used in the field trials in Senegal, but were not identical as the original seedlots were no  
3 longer available. The glasshouse trials enabled photosynthetic light curves to be determined  
4 under controlled conditions and water table effects on seedling development and biomass  
5 allocation to be evaluated. Seed was pre-screened for any surface deformity before being  
6 randomly allocated to treatments. To enhance germination, seeds were pre-treated by rubbing  
7 a small area of seed coat opposite the micropyle with fine sand paper and soaked in cold  
8 water until the seed had swelled.

### 9 *Light Curve Determination*

10 A randomized block design trial was set up in 2009 to estimate photosynthetic light curves  
11 for four month old saplings from 12 provenances. Eight blocks were set up with one tree per  
12 provenance in each block and light curves were estimated from one mature leaf per tree  
13 giving a total of ninety six light curves i.e. eight curves per provenance. However, data for  
14 three light curves were rejected- Bissiga (2) and Kigwe (1) due to a leaky chamber, which led  
15 to an unbalanced design for analysis (see below). Instantaneous WUE was also calculated as  
16 the ratio of evapotranspiration to carbon fixation. Light curves were determined using an LI-  
17 6400XP portable photosynthetic system (LICOR Environmental - UK Ltd, Cambridge, UK)  
18 and light was supplied by an internal red/blue LED light source (LI6400-02B). The leaf  
19 temperature was controlled at 28 °C; the maximum deviation was near 2 °C but was usually  
20 less than 1 °C during the course of curve determination. Ambient air was scrubbed of CO<sub>2</sub>,  
21 and CO<sub>2</sub> was controlled by addition from pressurized bottles at around 400 ppm; vapour  
22 pressure deficit (VPD) was kept constant at 1.8 kPa. Light response curves were generated  
23 using the LICOR photosynthesis software equations in LICOR application note 105 (Norman  
24 et al. 1992). Leaf area was determined from photographs of the leaves in the chamber,

1 analysed in Image J software (Rasband 1997-2011). Instantaneous WUE was calculated from  
2 the light curve data generated from PAR values ranging from 2000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  to 0  $\mu\text{mol m}^{-2}$   
3  $\text{s}^{-1}$ .

#### 4 *Water Table Effects*

5 A second randomized block design trial was set up in 2010 using the same 12 provenances  
6 planted in 10 cm diameter pots of 30 cm and 50 cm depths, representing two different depths  
7 to water source. There was one replicate of each provenance  $\times$  depth combination in each of  
8 eight blocks. All pots were located on a bench with watering system set up to soak capillary  
9 matting for 2 minutes 30 seconds every 2 hours from 07.00 - 18.00 h. Pots were filled with  
10 John Innes number 3 compost, with 3 cm of seed compost on top. Seeds were sown (on 1<sup>st</sup>  
11 June 2010) on the surface (one per pot) and covered lightly with horticultural sand. After  
12 seven weeks plants were destructively harvested and scored for survival, and measurements  
13 made of root and shoot biomass; this material was dried in a conventional oven at 60 °C for  
14 24 hrs. Leaf area and specific leaf area were estimated from a random sample of 3 mature  
15 leaves per tree. These leaves were then bulked for each provenance to determine  $\delta^{13}\text{C}$  value.  
16 Plants that failed or struggled to reach the water source (as indicated by either low root  
17 biomass or by dying before harvest) typically showed symptoms of water stress e.g. leaf loss.

#### 18 *Laboratory Analyses*

19 Dried leaf samples were ground by a ball mill to a fine powder and analysed at two isotope  
20 laboratories, the Life Sciences Mass Spectrometry Facility (LSMSF) at Lancaster, UK (for  
21  $^{13}\text{C}$  analysis) and the Stable Isotope Facility (SIF) at UC Davis, California, USA mainly for  $^{15}\text{N}$   
22 N but additional  $^{13}\text{C}$  data were also provided by this laboratory and used in the analyses. At  
23 the LSMSF laboratory,  $^{13}\text{C}$  analysis samples were weighed into tin capsules and combusted  
24 using a CarloErba elemental analyser. Resultant  $\text{CO}_2$  from combustion was analysed for  $\delta^{13}\text{C}$

1 using a Dennis Leigh technology isotope ratio mass spectrometer (IRMS). At the SIF,  
2 weighed samples in tin capsules were analysed using a PDZ Europa ANCA-GSL elemental  
3 analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd.,  
4 Cheshire, UK). Samples were combined with several replicates of at least two different  
5 laboratory standards previously calibrated against NIST Standard Reference Materials  
6 (IAEA-N1, IAEA-N2, IAEA-N3, USGS-40, and USGS-41). The long term standard  
7 deviation is 0.2 ‰ for  $^{13}\text{C}$  and 0.3 ‰ for  $^{15}\text{N}$ . All values were expressed relative to  
8 international standards V-PDB (Vienna PeeDee Belemnite) and air for carbon and nitrogen,  
9 respectively.

#### 10 *Statistical Analysis and Environmental Characterisation of Provenances*

11 As our sampling was unbalanced (i.e. 5 leaves in the wet and 10 in the dry), for the Senegal  
12 trials, we applied an unbalanced analysis of variance to the data using GenStat (version 13,  
13 VSN International Ltd., Hemel Hempstead, United Kingdom).

14 We also included in our analysis an assessment of life-form mainly to interpret BNF  
15 difference between fixing (*Acacia*) and non-fixing (all the other species) but also in WUE  
16 since there are likely to be differences in life form water use strategies. The life-forms used  
17 here are as follows: leguminous trees (*Acacia*) non-leguminous trees (trees), shrubs and  
18 herbaceous plants (herbs).

19 To account for any block effects tests for site, season, life-form and provenance effects were  
20 done once block effects had been accounted for i.e. on residual variance. In addition,  
21 provenance effects were only tested once variation that could be attributed to plasticity was  
22 removed (site, block and season). As reference material was collected outside the trial and to  
23 remove variation due to this these were treated as an additional block in the analysis (block  
24 5). The growth form factor used the classification *Acacia*, (i.e. *Acacia senegal* only), tree (all



1 other tree species), shrub (woody species < 5m), herb and grass. For the glasshouse studies a  
2 balanced analysis of variance was applied to the data again using GenStat and testing for any  
3 effects once the block effects were accounted for. For the Senegal data both tree height and  
4 laboratory ( $\delta^{13}\text{C}$  only) were initially included as covariates in the full model but were  
5 subsequently dropped from the analysis as they were found not to be significant.

6 To examine the links between the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  provenance results and ‘native’  
7 environmental conditions i.e. those at the place of origin of the different provenances, not  
8 those at the trial sites, we used summary climate data from LocClim (see Gommès et al.  
9 2004) and soil data from The Harmonized World Soil Database (HWSD)  
10 (FAO/IIASA/ISRIC/ISSCAS/JRC 2009) (see supplementary material, Table S2, for the  
11 variables included). These data were subjected to Principal Component Analysis (PCA) in  
12 Canoco 4.5 (ter Braak and Smilauer 2002). The isotope data were entered into the PCA  
13 model as supplementary variables. These are passively entered into the ordination but do not  
14 influence the ordination axes i.e. they are added post-analysis so that their relation to the  
15 other variables can be judged from the ordination diagram (see ter Braak and Smilauer  
16 2002). Correlation analysis was also performed to determine the relationship between the  
17 traits.

## 1 **Results**

### 2 *Senegal Trials*

#### 3 *Provenance $\delta^{13}C$*

4 Block was a significant term in the model, but analysis once this was accounted for, revealed  
5 clear effects of season, site, life-form and provenance in terms of  $\delta^{13}C$  (Table 2).  $\delta^{13}C$  values  
6 were lower in the dry season compared to the wet and values from the trial at Dahra tended to  
7 be lower than those from Bambey (Figure 2). There were also differences in life-form with  
8 *Acacia* tending to show lower values than herbs, shrubs or other tree species, particularly in  
9 the dry season. Provenance level differences were more evident in the dry season and  $\delta^{13}C$   
10 values correlated with gum production: lower values were correlated with higher gum  
11 production (Figure 2). This relationship appeared to be stronger in the dry season although  
12 sample size was lower in the dry season data.

#### 13 *Provenance $\delta^{15}N$ and $\%N$*

14 Again, although block was a significant term in the model, there were similar seasonal, site,  
15 life-form and provenance effects for  $\delta^{15}N$  values (Table 2).  $\delta^{15}N$  values were lower in the wet  
16 season compared to the dry and values from the trial at Dahra tended to be slightly higher  
17 than those from Bambey (Figure 3). Life-form also shows differences with *Acacia* tending to  
18 have higher values in the dry season but similar values to herbs and other trees in the wet  
19 season. Provenance level differences were more evident in dry season. In contrast to the  $\delta^{13}C$   
20 data there was no correlation between gum production and  $\delta^{15}N$  (Figure 3). Results showed  
21 high variability of  $^{15}N$  natural abundance values within and among provenances with a  
22 significant effect ( $P < 0.05$ ) of season (dry & wet) and location of the experiment (Dahra &  
23 Bambey). At Dahra, the  $\delta^{15}N$  values among all provenances (pooled) ranged from 2.33 ‰ to

1 11.22 ‰ during the dry season and from 4.45 ‰ to 8.23 ‰ during the wet season. The  $\delta^{15}\text{N}$   
2 values for reference plants ranged from 3.86 ‰ (*Combretum glutinosum* Perr., tree) to 6.62  
3 ‰ (*B. aegyptiaca*, tree) for dry season and from 3.16 ‰ (*Bauhinia rufescens*, Lam., shrub) to  
4 8.47 ‰ (*Achyranthes argentea*, herb) for wet season. At Bambey, where only wet season  
5 samples were analysed; the  $\delta^{15}\text{N}$  values ranged from 1.33 ‰ (*A. senegal*, Kirane provenance)  
6 to 8.47 ‰ (*A. senegal*, Sudan provenance [=Kordofan]). All provenances showed lower  $\delta^{15}\text{N}$   
7 values at Bambey (3.81 ‰ to 5.05 ‰) than Dahra (6.11 ‰ to 6.41 ‰). The  $\delta^{15}\text{N}$  values for  
8 reference plants ranged from 2.13 ‰ (*Zizyphus mauritiana* Lam., shrub) to 7.32 ‰ (*B.*  
9 *aegyptiaca*, tree).  $\delta^{15}\text{N}$  values for the grasses, *Brachiara disticophylla* Stapf. and  
10 *Andropogon gayanus* Kunth., sampled during the wet season in Bambey were 4.29 ‰ and  
11 4.99 ‰, respectively. In Dahra, the mean % N values for *A. senegal* provenances were  
12 generally similar for both wet (4.2 % - 4.6 %) and dry (4.1 % - 4.4 %) season. Reference  
13 plants showed slightly higher mean % N values in wet than dry season (4.0 % vs. 3.3 %). In  
14 Bambey, mean % N values ranged from 3.4 % to 4.3 % for *A. senegal* provenances, while  
15 that of reference plants was significantly lower (2.4 %).

16 In order to be able to estimate BNF by the  $^{15}\text{N}$  natural abundance method, one of the most  
17 important criteria is that  $\delta^{15}\text{N}$  values of the putative  $\text{N}_2$ -fixing plant ( $\delta^{15}\text{N}_{\text{leg}}$ ) are between  
18  $\delta^{15}\text{N}_{\text{fix}}$  and  $\delta^{15}\text{N}_{\text{ref}}$  (Shearer & Kohl, 1986). Among all the analysed reference plants, only *B.*  
19 *aegyptiaca* could be reliably used as a suitable non- $\text{N}_2$ -fixing reference plant ( $\delta^{15}\text{N}_{\text{ref}}$ ) during  
20 the wet season. In Bambey, the only sampled *Balanites aegyptiaca* tree had  $\delta^{15}\text{N}$  value of  
21 7.32 ‰, while the nearest *A. senegal* provenance (Kordofan) had a mean  $\delta^{15}\text{N}$  value of 5.07  
22 ‰, indicating a difference of > 2 ‰ between the putative  $\text{N}_2$ -fixing and non- $\text{N}_2$ -fixing  
23 reference plants (Table S3). In Dahra the difference in  $\delta^{15}\text{N}$  values between *B. aegyptiaca*  
24 and *A. senegal* was < 1 ‰ during the wet season, while *A. senegal* provenances were more

1 enriched than any of the reference plants in the dry season. Estimates of BNF based on the  
2 wet season data with *B. aegyptiaca* were either low or negligible in Dahra (< 15 %), with  
3 Soderia (Ethiopia) provenance showing virtually no fixation. On the other hand, BNF  
4 estimates in Bambey ranged from 28 % for Ngane provenance to 38 % for Kankoussa  
5 provenance.

#### 6 *Variation of $\delta^{13}C$ Abundance Within and Among Kankoussa Provenance Trees*

7 As predicted, the block effect was also present within the Kankoussa  $\delta^{13}C$  data (Table 2).  
8 However, once block effect was accounted for there were similar seasonal effects as in the  
9 whole provenance analysis as well as a significant effect of tree. Seasonal effects followed a  
10 similar pattern as above with wet season values tending to be greater than dry season (Figure  
11 4). Among tree variability was as great as between provenances indicating that individual  
12 strategies also vary; one tree reversed the predominant trend having a higher mean  $\delta^{13}C$  value  
13 in the dry compared to the wet season (Figure 4).

14

1 *Glasshouse Trials*

2 *Light Curves*

3 A range of mean light curve parameter values was evident (Table 3). However, only the  
4 response to light saturation showed significant variation among provenances (see  
5 supplementary material Table S4). The variation in maximum assimilation rate ( $A_{max}$ ) was  
6 not significant ( $p=0.09$ ) but is mentioned here as a significant correlation was detected (see  
7 below). Mean light saturation ranged from approximately  $1100 \mu\text{mol m}^{-2}$  (Fallatu Forest) to  
8  $2500 \mu\text{mol m}^{-2}$  (Kibwezi and Ouagadougou). Mean maximum assimilation rates ranged from  
9 approximately  $30 \mu\text{mol s}^{-1} \text{m}^{-2}$  (Mali) to  $60 \mu\text{mol s}^{-1} \text{m}^{-2}$  (Ouagadougou) (see Table 3 and  
10 correlations with biomass below).

11 *Water Table*

12 In the 30 cm pots Kibwezi had the largest mean leaf area (and standard error) while Bissiga  
13 had the largest specific leaf area (Table 4). Ouagadougou had the smallest leaf area although  
14 this was from one tree (the other surviving tree had no leaves). Dara had the largest mean root  
15 biomass but both India ICRAF and Kigwe had more than double the shoot biomass of the  
16 other provenances showing distinct provenance differences. A negative correlation was noted  
17 between  $A_{max}$  and survival in 30 cm pots ( $R = -0.63$ ;  $p = 0.027$ ). Cameroon had the highest  
18 level for instantaneous WUE and Kiembara the lowest but this measure did not correlate to  
19 any of the other estimated values including  $\delta^{13}\text{C}$ .

20 In the 50 cm pots India ICRAF had the largest leaf area and again Ouagadougou had the  
21 lowest but again this was based on only one tree. Again, Bissiga had the largest specific leaf  
22 area but in contrast to the 30 cm pots India ICRAF had the largest root and shoot biomass.  
23 Also in common with above both India and Kigwe had shoot biomass more than double that

1 of the other provenances. A negative correlation with light saturation and survival in 50 cm  
2 pots ( $R = -0.63$ ;  $p = 0.028$ ) was also detected.

3 There was no detectable difference between pot treatments in bulked provenance  $\delta^{13}\text{C}$ .  
4 However, there was a significant correlation between the amount of biomass and the  $\delta^{13}\text{C}$  in  
5 the 50 cm pot but no correlation in the 30 cm pots (Table 4). None of the estimated leaf  
6 variables measured correlated with  $\delta^{13}\text{C}$  and there were no differences in leaf area or specific  
7 leaf area between pot treatments (data not shown). However, root biomass was positively  
8 correlated with  $\delta^{13}\text{C}$  in 50 cm pots ( $R = 0.84$ ;  $p = 0.005$ ), and light saturation was positively  
9 correlated with  $\delta^{13}\text{C}$  in 30 cm pots ( $R = 0.84$ ;  $p = 0.002$ ).

#### 10 *Environmental Characterisation of Provenances*

11 The PCA ordination showed some potential links to environmental characteristics from  
12 provenance home locations (Figure 5).  $\delta^{13}\text{C}$  value positively correlated with wind speed and  
13 some soil characters, particularly organic matter (Top OM, Figure 5) and water content  
14 (AWC, Figure 5). Variation in  $\delta^{13}\text{C}$  values from the glasshouse experiment and field trial in  
15 Senegal were in opposing directions. Light saturation was negatively correlated with  
16 irradiance ( $R = -0.63$ ;  $p = 0.027$ ) and potential evapotranspiration ( $R = -0.63$ ;  $p = 0.028$ ) and  
17 positively correlated to rainfall ( $R = 0.67$ ;  $p = 0.017$ ). Variation in wind was also negatively  
18 correlated with weighted mean gum production ( $R = -0.85$ ;  $p = 0.033$ ).  $\delta^{15}\text{N}$  was negatively  
19 correlated to some soil characteristics particularly bulk density and climate in terms of air  
20 temperature. Figure 5 would also suggest that water content and potential evapotranspiration  
21 may also be important factors for  $\delta^{15}\text{N}$ .

## 1 **Discussion**

### 2 *Water Use Efficiency*

3 We used  $\delta^{13}\text{C}$  as a surrogate for WUE and found clear provenance level differences and  
4 significant within provenance variation. In combination with the results of PCA, this  
5 demonstrates a genetic basis for eco-physiological differences among and within  
6 provenances, probably as a result of adaptation to local environments at their geographic sites  
7 of origin. While, Raddad and Luukkanen (2006) found that values of  $\delta^{13}\text{C}$  were less negative  
8 in the *A. senegal* provenances from sandy soils compared to those from clay soils. This  
9 suggests that provenances from sandy soils displayed conservative water use. Similarly,  
10 Newton et al. (1996) found that *Acacia tortilis* (Forssk.) Hayne trees growing on drier sites  
11 displayed relatively higher WUE than those on a wetter site. Likewise, Midgley et al. (2004)  
12 found evidence to suggest higher leaf-level WUE in dominant woody species (including  
13 *Acacia* spp.) from drier savannah sites. As such, our results add to the growing evidence that  
14 variation in WUE occurs within species as well as between species.

15 In addition, we show that *A. senegal*, may exhibit different WUE strategies at different life  
16 stages. Results from our glasshouse trial on young plants concur with Raddad & Luukkanen  
17 (2006), in that provenances from sites with low water availability showed conservative water  
18 use. Whilst our results from the mature trees in the Senegal trials suggest trees from dry sites  
19 were less water use efficient, they are in contrast to reports of other studies with *Acacia* and  
20 woody species in dryland savannas (e.g. Newton et al. 1996; Midgley et al. (2004). Although  
21 experimental artefacts cannot be ruled out, it is possible that the *A. senegal* has a plasticity in  
22 WUE that maximises water use efficiency whilst young and shallow-rooted, but relaxes water  
23 control once roots are deeper and able to access groundwater (Ong et al. 2002; Otieno et al.  
24 2005a; Otieno et al. 2005b). Nonetheless, field comparisons will be required to compare

1 mature and young trees in common environments to establish if this observation is robust (c.f.  
2 Gebrekirstos et al. 2006).

3 Interpreting foliar  $\delta^{13}\text{C}$  in terms of plant water use efficiency (WUE) relies on the  
4 assumption that it is stomatal conductance and not carbon assimilation that varies. We have  
5 no direct evidence of whether the provenance differences in WUE are a result of stomatal  
6 conductance or carbon assimilation, as our  $\delta^{13}\text{C}$  values did not correlate with instantaneous  
7 WUE. Departures from the relationship have been reported (Marron et al. 2005; Monclus et  
8 al. 2006; Monneveux et al. 2006), however, our photosynthesis measurements were made on  
9 different plants and at a different time period to those for  $\delta^{13}\text{C}$ . Ultimately WUE at the leaf  
10 level depends on stomatal regulation, evaporative demand and carbon fixation. WUE and  
11  $\delta^{13}\text{C}$  can vary independently (Seibt et al. 2008) because as stomata respond, depending on  
12 whether there is isohydric (tight stomatal control, resulting in a minimum, threshold leaf  
13 water potential for stomatal closure) or anisohydric (less strict control, with no discernible  
14 threshold) responses, the influence of vapour pressure deficit is modified such that further  
15 increases in vapour pressure deficit after a certain value do not influence WUE (Maseda and  
16 Fernández 2006).

17 We also found differences in  $\delta^{13}\text{C}$  values that correlate with gum production. The Sudanese  
18 provenance produced the most consistent gum supply and was characterised by a lower  $\delta^{13}\text{C}$   
19 signal. This largely agrees with the findings of Raddad & Luukkanen (2006) who found a  
20 similar relationship with gum yield. However, Gum yield is known to vary according to a  
21 number of factors such as climate and tapping date and method (Ballal et al. 2005a; Ballal et  
22 al. 2005b; Raddad et al. 2006). Reliable data on gum arabic yield and gum yield trends are  
23 generally lacking (Ballal et al. 2005a and references therein), therefore, more comprehensive  
24 datasets and detailed examination are required before firm conclusions can be drawn.



## 1 *Biological Nitrogen Fixation*

2 Estimates of BNF by *A. senegal* during the wet season were within the range reported for the  
3 species in a four year-old provenance trial in the Blue Nile region, Sudan (cf. 24-61%,  
4 Raddad et al. 2005), and comparable to those from natural populations of *A. senegal* at  
5 Baringo, Kenya, in the Rift Valley (cf. 33 -39%, Isaac et al. 2011). However, our estimates,  
6 particularly in Bambey, should be treated with caution because they were based on a single  
7 non-N<sub>2</sub>-fixing reference tree, *B. aegyptiaca*, which had a  $\delta^{15}\text{N}$  value higher than the mean  
8 values of the *A. senegal* provenances (Table S3). The  $\delta^{15}\text{N}$  values of the rest of the non-N<sub>2</sub>-  
9 fixing reference plants (other trees, shrubs and herbs), including their means, were  
10 indistinguishable from  $\delta^{15}\text{N}$  values for *A. senegal* (Figure 3). The  $\delta^{15}\text{N}$  values of *A. senegal*  
11 trees at Dahra during the dry season were highly enriched, and mostly surpassed those of the  
12 reference plants, resulting in negative or non-significant BNF estimates (Table S3). Similar  
13 null estimates have been observed in natural *A. senegal* trees, which were attributed to low P  
14 soils (Isaac et al. 2011), and, in *Acacia* species in dry woodland savannas, constraints on  
15 water availability (Handley et al. 1994). In our study, high temperatures and moisture stress  
16 may be responsible for the null estimates at Dahra during the dry season. Such conditions are  
17 common during dry seasons in the Sahelian region and may constrain legume root-nodule  
18 formation and function (Hungria and Vargas 2000; Rasanen and Lindstrom 1999).

19 However, it is also possible that, particularly in the dry season, there is an alternative N  
20 source available. Although soil was not analysed in this study, the high and variable leaf N  
21 concentrations in both putative N<sub>2</sub>-fixing *A. senegal* provenances and non-N<sub>2</sub>-fixing reference  
22 plants suggest different sources of plant available soil N. As suggested for the water relations  
23 above, the mature trees may tap into the widespread N source below the water table found in  
24 the interstitial waters in this region (Deans et al. 2005; Edmunds and Gaye 1997). *Acacia*

1 species are certainly known to access water resources from this depth (Otieno et al. 2005b),  
2 and may well make use of available N at the same time. Alternatively, organic matter may be  
3 a possible source (see Näsholm et al. 2009). For example, Bernhard-Reversat & Poupon  
4 (1980) found mineralization of organic N in the topsoil to be relatively important directly  
5 under trees suggesting N cycling to be an important source of plant available N, with the  
6 process occurring at the beginning of the rainy season.

### 7 *Eco-physiological Strategies in Acacia senegal*

8 Our results indicate that variation in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in *A. senegal* has both genetic and age-  
9 related components. Among and within provenance differences, probably arise as a result of  
10 adaptation to local environments in the region of origin. This suggests that selection for gum  
11 production in *A. senegal* might also be successful; it is certainly likely that some passive  
12 selection has already occurred particularly in Sudan populations. However, both plantation  
13 and provenance location environmental conditions are likely to be important in assessing  
14 selection criteria. In addition, our results suggest that high gum yield is associated with plants  
15 that have lower WUE and so there is potential for conflict in selection criteria. Experience  
16 has shown that improvements in leaf-level water use efficiency may not always translate into  
17 higher crop water-use efficiency or yield (Condon et al. 2004). Nevertheless, the level of  
18 environmental variability, the between and within population variation reported here and the  
19 differences in age class, suggest that there are prospects for genetic improvement. Given *A.*  
20 *senegal*'s potential poverty alleviation importance, especially in the dry season, this should be  
21 a future research priority.

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1

2

## 1 Tables

2 Table 1: Origins and environmental characteristics of *Acacia senegal* provenances used in the  
 3 field and glasshouse studies S = Senegal trial; G= Glasshouse Trial; \* indicates wild collected  
 4 seed. Gum yield data are shown for provenances for which they were available (based on data  
 5 for 2001). PET = potential evapotranspiration.

Country	Provenance	Longitude (decimal degrees)	Latitude (decimal degrees)	Alt (masl)	Mean monthly rain (mm)	Mean monthly PET (mm)	Mean Temp (°C)	Exp. Trial	Mean Provenance gum Prod. (g yr <sup>-1</sup> )
Sudan	Kordofan	29.58	12.73	620	32.0	205.0	26.0	S	244
Mali	Aïte	-11.65	15.08	80	45.6	151.8	30.3	S	194
Mauritania	Kankoussa	-11.45	15.93	80	27.2	179.2	29.5	S	190
Chad	Tourba	15.3	12.82	280	29.3	172.5	29.9	S	156
Ethiopia	Sodera	39.38	8.4	1500	64.8	114.5	20.7	S	136
Mali	Kirane	-10.25	15.38	140	25.5	203.8	28.9	S	127
Senegal	Diaménar	-15.9	16	20	25.2	197.2	25.4	S	105
Niger	Karofane	-6.18	14.3	280	44.3	163.7	27.4	S	94
India	Indie 50/60	79.52	26.32	120	87.1	146.4	25.8	S	91
Senegal	Ngane	-16.2	14.13	0	49.3	170.2	28.0	S	79
Burkina Faso	Bissiga	-0.53	12.43	280	62.3	181.9	28.5	G*	
Cameroon	Cameroon	14.23	10.25	440	65.8	190.8	27.9	G*	
Burkina Faso	Dara	-0.18	14.33	260	32.4	157.9	28.9	G*	
Sudan	Fallatu Forest	30.14	13.1	600	26.5	205.0	27.3	G	
India	India ICRAF	73.14	26.72	260	33.5	159.3	26.5	G	
Kenya	Kibwezi	38.07	-2.21	700	56.2	126.1	24.0	G	
Burkina Faso	Kiembara	-2.72	13.25	300	49.0	160.4	28.3	G*	
Tanzania	Kigwe	35.48	-6.1	940	44.7	154.3	23.6	G	
Burkina Faso	Kirbou	-2.07	13.26	300	50.1	160.4	28.1	G*	
Mali	Mali	-11.43	14.45	40	52.7	161.8	29.3	G*	
Burkina Faso	Ouagadougou	1.5	12.38	300	60.8	160.9	28.9	G*	
Tanzania	Wangingombe	34.63	-8.85	1320	67.7	129.0	22.2	G	
Senegal	Dahra Trial	-15.43	15.35	60	32.8	149.7	27.2		
Senegal	Bambey Trial	-16.47	14.71	20	42.8	176.0	26.5		

6

1 Table 2: Results of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analyses of variation of water use efficiency and biological  
 2 nitrogen fixation in *Acacia senegal*. The top two sections give results for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$   
 3 analysis of variance using an unbalanced design for the *A. senegal* leaf samples from the  
 4 trials at Dahra and Bambey in Senegal. The bottom section is the results of a balanced  
 5 analysis of variation for the  $\delta^{13}\text{C}$  results for Kankoussa provenance at Dahra only.

Variate	Factor	d.f.	s.s.	m.s.	v.r.	F pr.	
$\delta^{13}\text{C}$	Block	4	292.1	73.0	45.5	<0.001	
	Season	1	123.1	123.1	76.7	<0.001	
	Site	1	7.5	7.5	4.7	0.031	
	Life-Form	2	75.6	37.8	23.5	<0.001	
	Provenance	10	34.0	3.4	2.1	0.021	
	Residual	837	1343.8	1.6			
	Total	855	1876.2	2.2			
$\delta^{15}\text{N}$	Block	4	83.6	20.9	11.6	<0.001	
	Season	1	351.5	351.5	194.7	<0.001	
	Site	1	163.8	163.8	90.8	<0.001	
	Life-Form	2	17.2	8.6	4.8	0.009	
	Provenance	10	36.2	3.6	2.0	0.033	
	Residual	304	548.7	1.8			
	Total	322	1201.0	3.7			
$\delta^{13}\text{C}$	Block	3	6.9	2.3	7.9	<0.001	
	Dahra	Season	1	36.6	36.6	125.5	<0.001
	Kankoussa	Tree	13	26.3	2.02	6.9	<0.001
	Residual	267	77.9	0.29			
	Total	284	147.8	0.52			

6

1 Table 3: Mean photosynthesis parameters (SD) derived from light curves of 12 provenances  
 2 of *Acacia senegal*. LS = light saturation point, LC = light compensation point, AQE =  
 3 quantum yield,  $A_{max}$  = the assimilation rate at light saturation,  $R_d$  = dark respiration and  
 4 IWUE = instantaneous water use efficiency; n= 8 plants per provenance.

Provenance	LS ( $\mu\text{mol m}^{-2}$ )	LC ( $\mu\text{mol m}^{-2}$ )	AQE	$A_{max}$	$R_d$	IWUE
Bissiga	2029.33 (456.80)	13.22 (1.95)	0.21 (0.06)	41.37 (9.98)	-2.43 (0.42)	3.29 (0.18)
Maroua	1531.39 (489.34)	19.67 (1.70)	0.15 (0.03)	37.52 (8.38)	-2.68 (0.26)	3.46 (0.16)
Dara	1228.18 (175.75)	17.57 (2.69)	0.13 (0.02)	34.09 (5.35)	-2.31 (0.37)	3.41 (0.18)
Fallatu Forest	1116.04 (188.55)	14.93 (1.81)	0.14 (0.01)	33.73 (5.92)	-1.92 (0.25)	3.37 (0.15)
India ICRAF	1558.49 (455.77)	16.65 (3.64)	0.20 (0.05)	32.05 (7.68)	-2.19 (0.28)	3.21 (0.49)
Kibwezi	2509.45 (623.26)	16.94 (2.62)	0.17 (0.03)	43.56 (6.60)	-2.43 (0.30)	2.83 (0.17)
Kiembara	1559.02 (305.85)	14.00 (2.27)	0.17 (0.04)	42.73 (10.03)	-2.07 (0.34)	2.31 (0.13)
Kigwe	1424.68 (347.15)	19.45 (1.89)	0.14 (0.02)	38.45 (11.52)	-2.47 (0.26)	2.94 (0.21)
Kirbou	1309.33 (282.26)	12.46 (1.46)	0.16 (0.03)	32.68 (5.68)	-1.88 (0.29)	2.86 (0.22)
Mali	1612.53 (268.79)	16.14 (2.46)	0.19 (0.02)	30.43 (3.77)	-2.51 (0.24)	3.02 (0.12)
Ouagadougou	2561.98 (813.77)	14.31 (2.38)	0.20 (0.04)	60.25 (13.55)	-2.31 (0.25)	3.26 (0.20)
Wangingombe	2810.52 (518.97)	14.74 (1.43)	0.19 (0.02)	50.41 (8.44)	-2.60 (0.31)	3.11 (0.11)

5

1 Table 4: Estimated mean (SE) of leaf area (LA), specific leaf area (SLA), root and shoot  
 2 biomass and  $\delta^{13}\text{C}$  for water table glasshouse trial. The instrumental standard deviation for the  
 3  $\delta^{13}\text{C}$  values was 0.2537. \* indicates either missing data or unable to calculate. There was a  
 4 Pearson correlation coefficient of 0.854 between  $\delta^{13}\text{C}$  and root biomass and 0.627 with shoot  
 5 biomass ( $p < 0.05$ ) in 50 cm pots; Pearson correlation coefficient of 0.64 between survival and  
 6 root biomass ( $P < 0.05$ ) in 30 cm pots.

Pot Depth (cm)	Provenance	Survival	LA (mm <sup>2</sup> )	SLA (mm <sup>2</sup> g <sup>-1</sup> )	Root (g)	Shoot (g)	$\delta^{13}\text{C}$
30	Bissiga	4	11.57 (3.42)	0.33 (0.03)	4.02 (2.13)	3.88 (2.15)	-31.57
	Maroua	1	*	*	0.00 (*) <sup>a</sup>	0.08 (*)	*
	Dara	7	10.65 (1.08)	0.25 (0.01)	5.51 (1.27)	6.02 (1.29)	-31.92
	Fallatu Forest	3	13.59 (2.41)	0.22 (0.01)	3.15 (0.84)	5.01 (0.67)	-32.43
	India ICRAF	7	16.46 (1.97)	0.26 (0.04)	4.80 (0.92)	18.20 (4.38)	-31.85
	Kibwezi	3	24.95 (7.68)	0.24 (0.03)	4.22 (1.86)	8.33 (4.25)	-31.31
	Kiembara	7	12.67 (1.12)	0.26 (0.02)	3.21 (0.99)	3.99 (0.91)	-31.99
	Kigwe	2	17.51 (3.33)	0.20 (0.03)	4.66 (0.27)	22.90 (6.72)	-31.32
	Kirbou	7	11.59 (1.75)	0.26 (0.02)	3.68 (0.64)	4.68 (1.06)	-31.61
	Mali	6	14.61 (3.47)	0.26 (0.01)	3.43 (0.79)	5.38 (1.32)	-31.68
	Ouagadougou	2	7.84 (*)	0.25 (*)	0.92 (0.90)	0.89 (0.86)	-31.35
Wangingombe	0	*	*	*	*	*	
50	Bissiga	6	12.93 (2.43)	0.30 (0.05)	11.84 (3.09)	13.56 (4.09)	-31.06
	Maroua	0	*	*	*	*	*
	Dara	4	11.58 (1.37)	0.26 (0.03)	6.99 (2.48)	8.67 (2.81)	-32.13
	Fallatu Forest	6	10.65 (1.14)	0.27 (0.02)	10.63 (0.88)	18.23 (2.73)	-31.67
	India ICRAF	6	16.15 (1.09)	0.27 (0.02)	13.34 (2.17)	47.77 (4.27)	-31.15
	Kibwezi	5	13.72 (2.17)	0.29 (0.02)	5.19 (1.93)	9.56 (3.80)	-31.90
	Kiembara	5	12.53 (1.40)	0.26 (0.01)	9.55 (4.44)	10.49 (4.86)	-31.81
	Kigwe	5	10.57 (1.92)	0.18 (0.00)	11.48 (0.98)	42.07 (2.31)	-31.33
	Kirbou	6	11.11 (1.94)	0.28 (0.02)	8.02 (1.77)	9.36 (1.30)	-31.77
	Mali	6	9.93 (1.45)	0.27 (0.04)	8.35 (1.99)	11.34 (2.66)	-31.93
	Ouagadougou	1	9.90 (*)	0.24 (*)	5.26 (*)	5.51 (*)	-32.25
Wangingombe	1	10.30 (*)	0.37 (*)	3.66 (*)	6.13 (*)	-32.85	

7 <sup>a</sup> Root too small to determine biomass.

8

## 1 **Figure Captions**

2 Figure: 1 (a) *Acacia senegal* provenance locations used in the trials in Bambey and Dahra,  
3 Senegal (dark circles) and glasshouse in Edinburgh, UK (grey circles). (b) Map of Senegal  
4 with administrative regions and locations of the trials.

5 Figure 2: (a) Mean  $\delta^{13}\text{C}$  values (‰) in relation to season [please note that although we use the  
6 terminology ‘dry season’ here October is not really the dry season *per se* but the **beginning**  
7 of the dry season], site and provenance, sampled at Dahra (wet August and dry October 2009)  
8 and Bambey (August only). Error bars represent 95% confidence intervals. (b) Mean  $\delta^{13}\text{C}$   
9 values in relation to season and life form, error bars represent 95% confidence intervals at  
10 Dahra only. Life-forms are as follows: leguminous trees (*Acacia*) non-leguminous trees  
11 (trees), shrubs and herbaceous plants (herbs). (c) Correlation between mean  $\delta^{13}\text{C}$  values in  
12 2009 and mean gum production in 2001, 2007-2009. Linear line is for reference only;  
13 Pearson correlation coefficient = - 0.697, p value < 0.05.

14 Figure 3: (a) Mean  $\delta^{15}\text{N}$  values (‰) in relation to season [please note that although we use  
15 the terminology ‘dry season’ here October is not really the dry season *per se* but the  
16 **beginning** of the dry season], site and provenance; error bars represent 95% confidence  
17 intervals. REFD and REFB refer to non-leguminous reference plants at Dahra and Bambey  
18 respectively (i.e. includes all life-forms except *Acacia*) (b) Mean  $\delta^{15}\text{N}$  values in relation to  
19 season and life form at Dahra; error bars represent 95% confidence intervals. Life-forms are  
20 as follows: leguminous trees (*Acacia*) non-leguminous trees (trees), shrubs and herbaceous  
21 plants (herbs).

22 Figure 4:  $\delta^{13}\text{C}$  (‰) for the Kankoussa provenance at Dahra only, mean  $\delta^{13}\text{C}$  values in relation  
23 to season [please note that although we use the terminology ‘dry season’ here October is not  
24 really the dry season *per se* but the **beginning** of the dry season] and individual tree. Five  
25 leaves measured per tree, error bars represent 95% confidence intervals of the mean.

26 Figure 5: (a) Axes 1 and 2 of a principal components analysis on soil and climate data from  
27 HWSD and LocClim respectively;  $\delta^{13}\text{C}$  (‰) data area projected into the ordination diagram  
28 passively. The cumulative percentage variance of axes 1 and 2 was 76.0 and 85.2  
29 respectively. Some variables have been omitted from the diagram for clarity retaining those  
30 with the highest correlations with axes 1 and 2. (b) Axes 1 and 2 of a principal components  
31 analysis on soil and climate data from HWSD and LocClim respectively;  $\delta^{15}\text{N}$  (‰) data area  
32 projected into the ordination diagram passively. The cumulative percentage variance of axes  
33 1 and 2 was 56.1 and 81.7 respectively. Again, some variables have been omitted from the  
34 diagram for clarity using the same criteria.

Figure 1



(a)



(b)

Figure 2

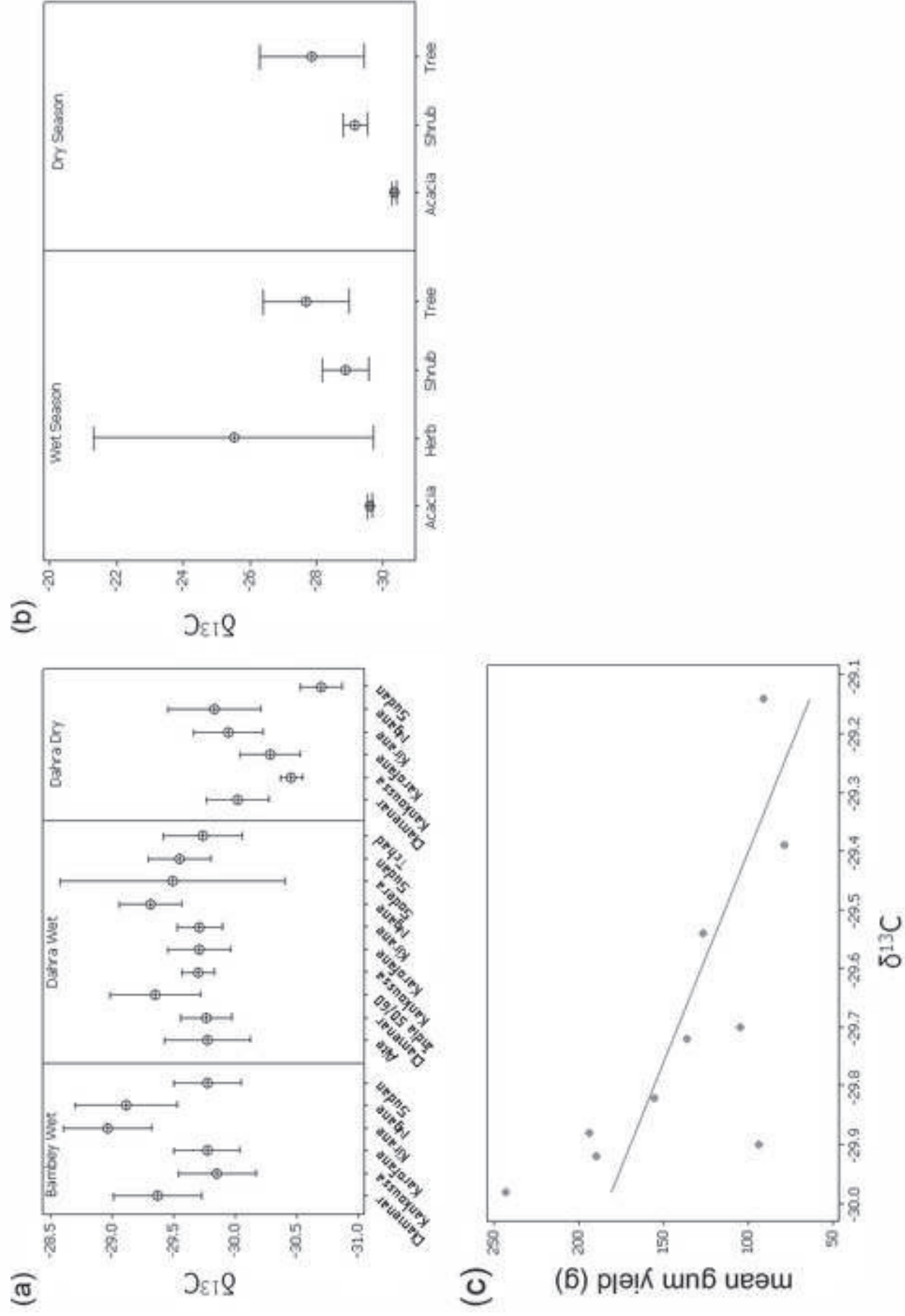
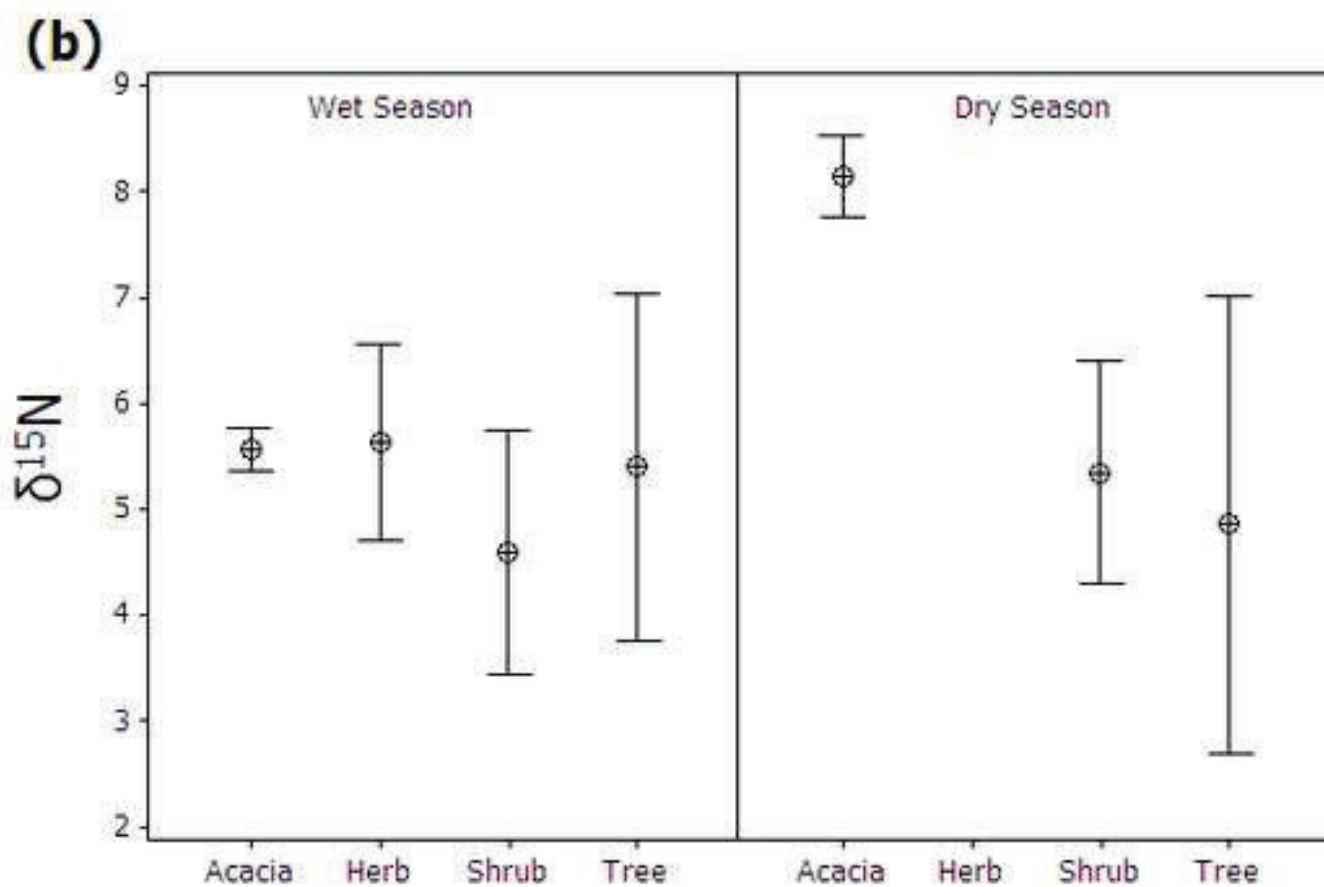
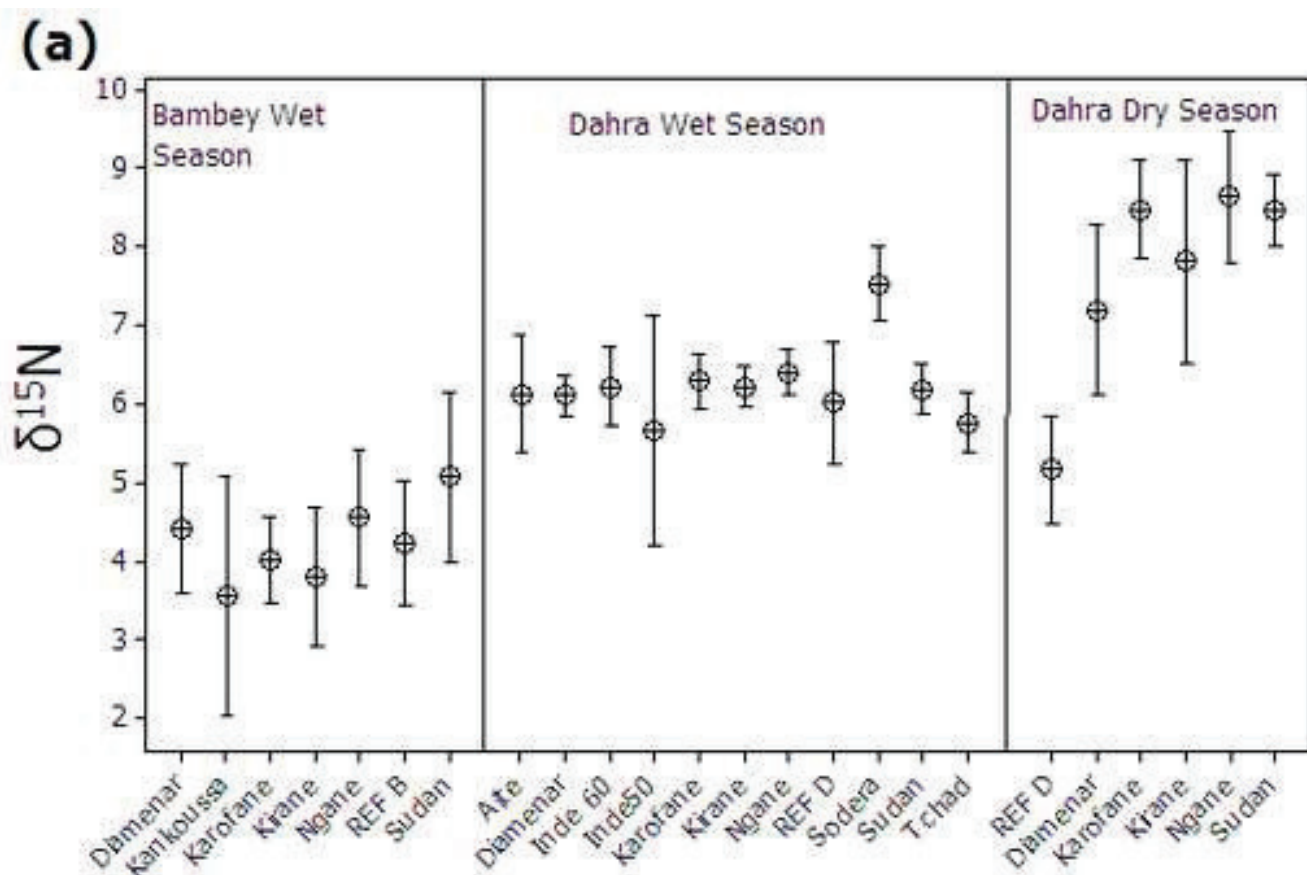




Figure 3



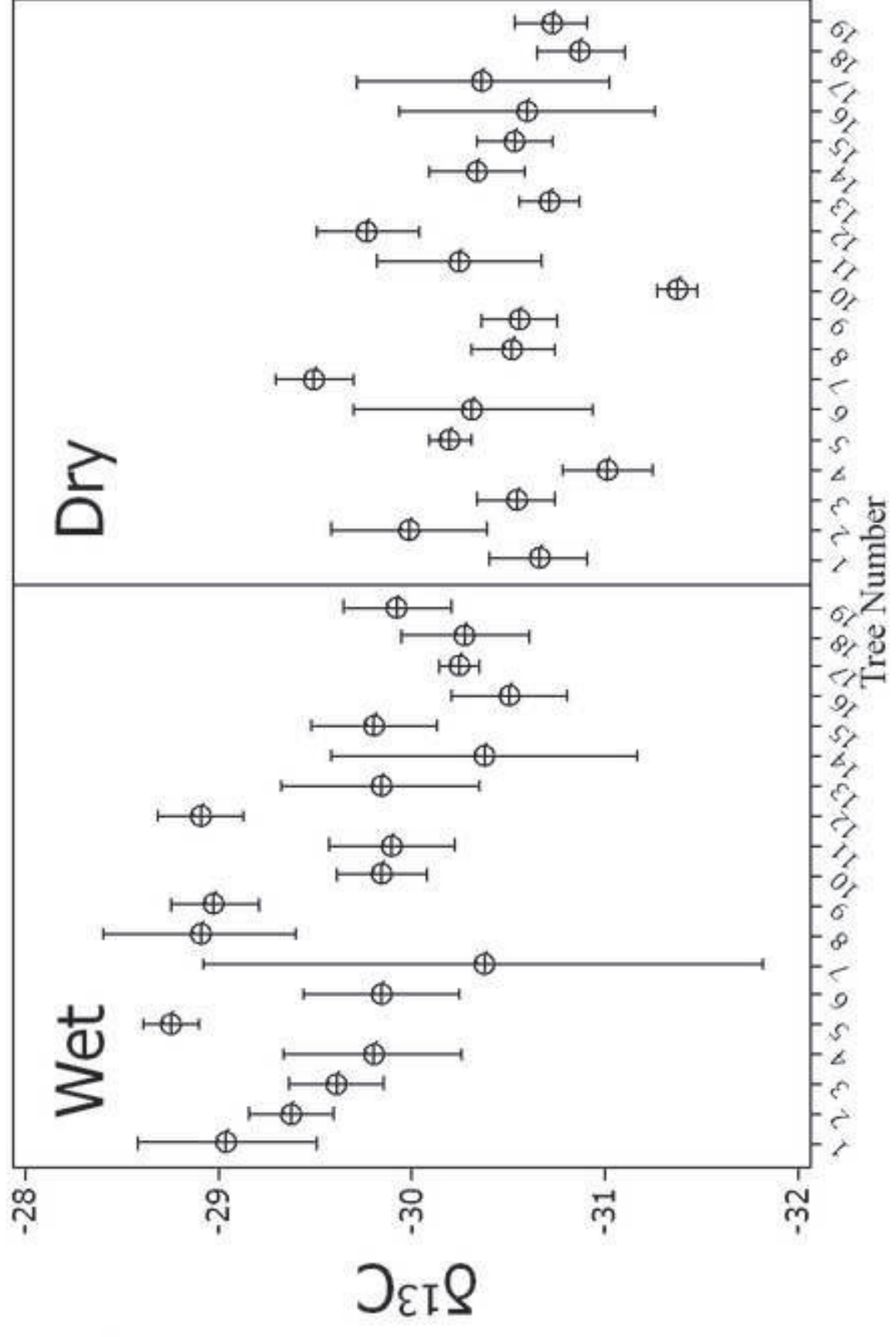
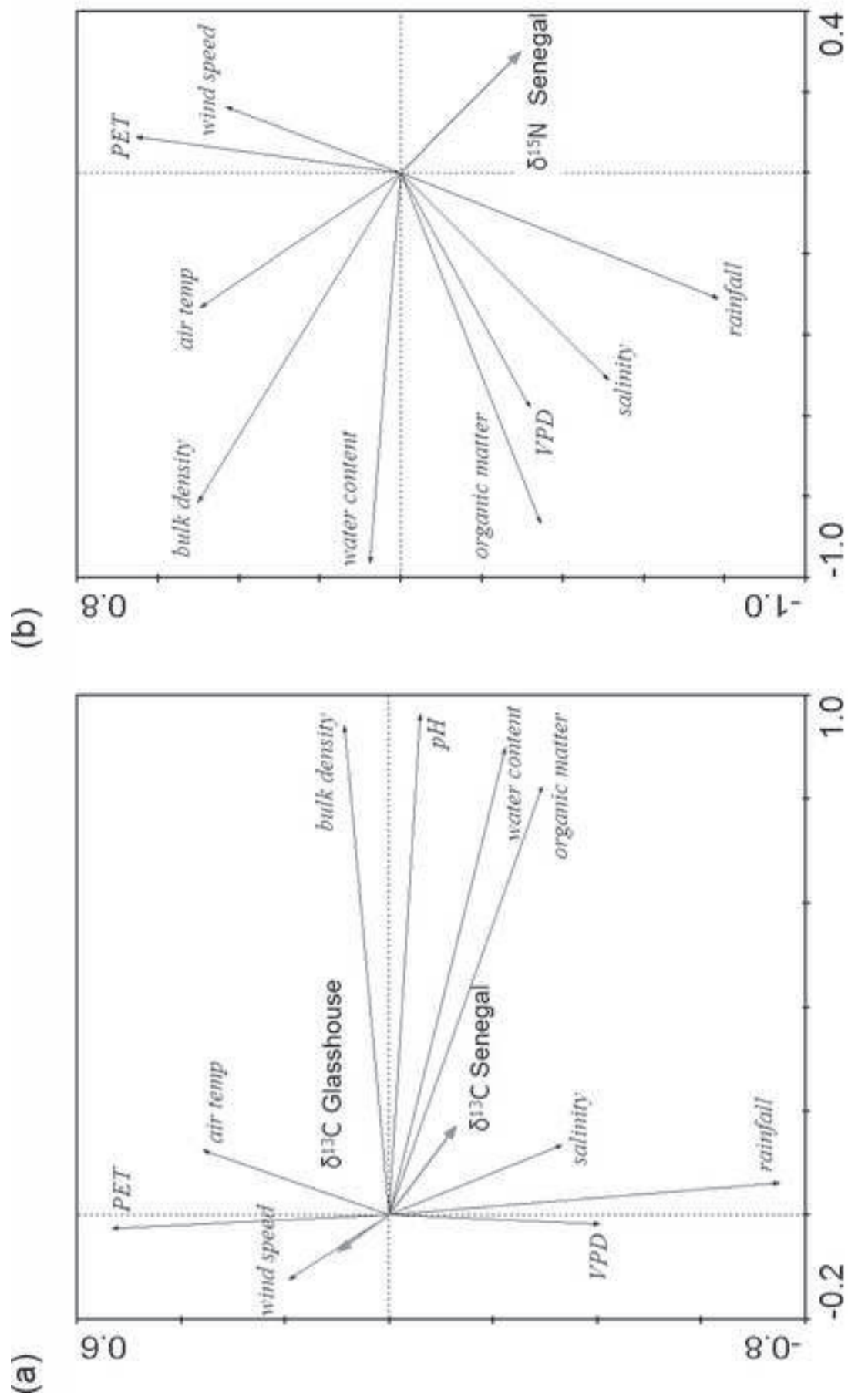


Figure 4

Figure 5



**Plant and Soil: Supplementary Material****Does geographic origin dictate ecological strategies in *Acacia senegal* (L.) Willd.?  
Evidence from carbon and nitrogen stable isotopes**

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Table S1: Mean tree height data for sites and provenances at the Senegal trials.

Site	Provenance	mean Height (m)	SE
Bambey	Aite	*	*
	Diamenar	2.62	0.27
	Kankoussa	2.93	0.27
	Karofane	2.93	0.22
	Kirane	3.60	0.24
	Ngane	3.31	0.25
	Sudan	3.38	0.22
Dahra	Aite	2.48	0.39
	Diamenar	3.65	0.19
	Inde 60	2.16	0.19
	Inde50	2.70	0.62
	Karofane	2.94	0.20
	Kirane	3.85	0.17
	Ngane	3.66	0.19
	Sodera	1.87	0.30
	Sudan	3.84	0.19
Tchad	2.50	0.36	

Table S2: Environmental variables used in the principal coordinates analyses with abbreviated codes used in Figure 5 and corresponding units.

Database	Environmental Variable	Figure 5 Code	Units
HWSD	water content	AWC	mm
	Topsoil Sand Fraction		%
	Topsoil Silt Fraction		%
	Topsoil Clay Fraction		%
	Topsoil Reference Bulk Density	Top Bulk	kg/dm <sup>3</sup>
	Topsoil Gravel Content		%
	Topsoil Organic Carbon	Top OM	% weight
	Topsoil pH	Top pH	in H <sub>2</sub> O
	Topsoil CEC (clay)		cmol/kg
	Topsoil CEC (soil)		cmol/kg
	Topsoil Base Saturation		%
	Topsoil TEB		cmol/kg
	Topsoil Calcium Carbonate		% weight
	Topsoil Gypsum		% weight
	Topsoil Sodicity (ESP)		%
	Topsoil Salinity (ECe)	Top Sal	dS/m
	Subsoil Sand Fraction		%
	Subsoil Silt Fraction		%
	Subsoil Clay Fraction		%
	Subsoil Reference Bulk Density	Sub Bulk	kg/dm <sup>3</sup>
	Subsoil Gravel Content		%
	Subsoil Organic Carbon		% weight
	Subsoil pH		in H <sub>2</sub> O
	Subsoil CEC (clay)		cmol/kg
	Subsoil CEC (soil)		cmol/kg
	Subsoil Base Saturation		%
	Subsoil TEB		cmol/kg
	Subsoil Calcium Carbonate		% weight
	Subsoil Gypsum		% weight
	Subsoil Sodicity (ESP)		%
	Subsoil Salinity (ECe)		dS/m
	LocClim	Potential evapotranspiration	PET
Annual rainfall		Rain	mm
Sunshine hours			hrs
Mean temperature		T mean	°C
Maximum temperature			°C
Minimum temperature			°C
Vapour pressure deficit		VPD	kPa
Standard deviation of rainfall			mm
Wind speed		Wind	km hr <sup>-1</sup>

Table S3: Provenance variation in BNF estimates of *Acacia senegal* provenances evaluated on foliar samples from Bambey and Dahra sites beginning (wet season, August 2009) of rains. Means ( $\pm$ standard error, SE) in leaf N (%) and the proportion of foliar N derived from BNF (% Ndfa). Estimates were based on *Balanites aegyptiaca* reference plants ( $\delta^{15}\text{N}_{\text{ref}}$ ) values: Bambey site, 7.32 ‰ ( $n = 1$ ); Dahra site,  $6.77 \pm 0.50$  ‰ ( $n = 3$ ).

Site	<i>n</i>	Provenance	% N $\pm$ SE	$\delta^{15}\text{N}(\text{‰})\pm$ SE	% Ndfa $\pm$ SE
Bambey	12	Kirane, Mali	3.40 $\pm$ 0.15	3.81 $\pm$ 0.40	35.7 $\pm$ 4.1
	15	Karofane, Niger	3.98 $\pm$ 0.10	4.03 $\pm$ 0.26	33.5 $\pm$ 2.6
	11	Diamenar, Senegal	4.05 $\pm$ 0.14	4.40 $\pm$ 0.37	29.7 $\pm$ 3.8
	7	Ngane, Senegal	3.38 $\pm$ 0.17	4.55 $\pm$ 0.36	28.1 $\pm$ 3.7
	15	Kordofan, Sudan	3.85 $\pm$ 0.12	5.07 $\pm$ 0.51	22.9 $\pm$ 5.2
	6	Kankoussa, Mauritania	4.26 $\pm$ 0.28	3.56 $\pm$ 0.60	38.2 $\pm$ 6.1
Dahra	18	Kirane, Mali	4.36 $\pm$ 0.09	6.22 $\pm$ 0.12	5.9 $\pm$ 1.3
	10	Aite, Mali	4.58 $\pm$ 0.07	6.13 $\pm$ 0.33	6.9 $\pm$ 3.6
	17	Karofane, Niger	4.48 $\pm$ 0.09	6.30 $\pm$ 0.16	5.1 $\pm$ 1.7
	20	Diamenar, Senegal	4.63 $\pm$ 0.11	6.11 $\pm$ 0.12	7.1 $\pm$ 1.3
	18	Ngane, Senegal	4.20 $\pm$ 0.11	6.41 $\pm$ 0.12	3.9 $\pm$ 1.3
	20	Kordofan, Sudan	4.38 $\pm$ 0.11	6.19 $\pm$ 0.16	6.3 $\pm$ 1.7
	6	Sodera, Ethiopia	3.36 $\pm$ 0.08	7.54 $\pm$ 0.19	-0.3 $\pm$ 2.0
	8	Jodhpur Inde60, India	4.20 $\pm$ 0.15	6.22 $\pm$ 0.21	5.9 $\pm$ 2.3
	5	Jodhpur Inde50, India	4.45 $\pm$ 0.27	5.66 $\pm$ 0.53	11.9 $\pm$ 5.7
9	Tourba, Chad	4.72 $\pm$ 0.15	5.77 $\pm$ 0.17	10.8 $\pm$ 1.8	

Table S4: Unbalanced ANOVA table for photosynthetic parameters, light saturation (top) and Amax (below).

Factor	d.f.	s.s.	m.s.	v.r.	F pr.
+ Block	7	25653685.	3664812.	2.75	0.013
+ Provenance	11	30692703.	2790246.	2.10	0.031
Residual	74	98546161.	1331705.		
Total	92	154892548.	1683615.		
Factor	d.f.	s.s.	m.s.	v.r.	F pr.
+ Block	7	7768.7	1109.8	2.61	0.018
+ Provenance	11	7981.7	725.6	1.71	0.088
Residual	74	31445.4	424.9		
Total	92	47195.8	513.0		

Figure S1: *Acacia senegal* provenance trial schematic for the Dahra Station, Provenances that were sampled for  $^{13}\text{C}$  and  $^{15}\text{N}$  are highlighted in yellow, note that in each provenance plot there were 25 trees randomly planted for each provenance. Also note that there was a 5 m buffer strip between blocks.

Block 4		Block 3		Block 2		Block 1	
Sodera	Djigueri	Inde 60	Ngane	Kirane	Sodera	Kirane	Sodera
Daiba	Kidira	Soudan	Kidira	Diamenar	Djigueri	Karofane	Aite
Burkina DI	Inde 60	Diamenar	Daiba	Kankoussa	Karofane	Soudan	Kankoussa
Diamenar	Ngane	Aite	Inde 50	Somo	Soudan	Djigueri	Diamenar
Kankoussa	Aite	Bissiga	Tchad	Aite	Pakistan	Pakistan	Somo
Somo	Inde 50	Sodera	Karofane	Kidira	Tchad	Burkina DI	Ngane
Tchad	Karofane	Djigueri	Kirane	Daiba	Burkina DI	Bissiga	Inde 60
Bissiga	Soudan	Kankoussa	Somo	Ngane	Inde 50	Tchad	Kidira
Pakistan	Kirane	Pakistan	Burkina DI	Inde 60	Bissiga	Inde 50	Daiba

Figure S2:  $\delta^{13}\text{C}$  values for leaves of *Rumex acetosa* L. dried in microwave oven and conventional oven; error bars represent instrumental SD.

