



Supplement of

Speculations on the application of foliar ^{13}C discrimination to reveal ground-water dependency of vegetation and provide estimates of root depth and rates of groundwater use

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Supplementary Material

Table S1: List of species, their functional types, $\Delta^{13}\text{C}$ and calculated intrinsic WUE_i (PFT; plant functional type codes: BIT= broad leaf tree, NIT= needle leaf tree and S=shrub)

Species	Site Code ¹	PFT	$\Delta^{13}\text{C}$ (‰)	Calculated WUE_i
<i>Acacia aneura</i>	AMU	NIT	19.71±0.21	78.67
<i>Acacia melanoxylon</i>	WR	BIT	22.46±0.46	49.00
<i>Acmena graveolens</i>	CT	BIT	21.96±0.31	54.37
<i>Alphitonia whitei</i>	RC	BIT	24.81±0.58	23.63
<i>Alstonia muelleriana</i>	RC	BIT	23.42±0.4	38.58
<i>Alstonia scholaris</i>	CT	BIT	22.76±0.36	45.75
<i>Anopterus glandulosus</i>	WR	S	25.58±0.44	15.34
<i>Argyrodendron peralatum</i>	CT	BIT	24.89±0.26	22.75
<i>Atherosperma moschatum</i>	WR	BIT	22.57±0.85	47.80
<i>Cardwellia sublimis</i>	CT	BIT	22.46±0.58	48.92
<i>Castanospermum australe</i>	CT	BIT	22.04±0.4	53.48
<i>Ceratopetalum succirubrum</i>	RC	BIT	26.52±0.13	5.21
<i>Corymbia terminalis</i>	AMU	BIT	19.89±0.29	76.65
<i>Cryptocarya mackinnoniana</i>	CT	BIT	24.59±0.12	26.02
<i>Daphnandra repandula</i>	RC	BIT	23.69±0.57	35.69
<i>Doryphora aromatica</i>	RC	BIT	25.41±0.88	17.19
<i>Dysoxylum papuanum</i>	CT	BIT	20.83±0.44	66.59
<i>Elaeocarpus grandis</i>	CT	BIT	22.34±0.1	50.30
<i>Endiandra leptodendron</i>	CT	BIT	26.86±0.6	1.51
<i>Eucalyptus amplifolia</i>	CP	BIT	21.02±0.31	64.53
<i>Eucalyptus camaldulensis</i>	AMU	BIT	20.28±0.35	72.43
<i>Eucalyptus clelandii</i>	GWW	BIT	17.68±0.17	100.56
<i>Eucalyptus dumosa</i>	CM	BIT	19.01±0.42	86.15
<i>Eucalyptus fibrosa</i>	CP	BIT	23.24±0.32	40.57

Species	Site Code ¹	PFT	$\Delta^{13}\text{C}$ (‰)	Calculated WUE _i
<i>Eucalyptus miniata</i>	LF	BIT	21.86±0.07	55.39
<i>Eucalyptus moluccana</i>	CP	BIT	21.48±0.24	59.50
<i>Eucalyptus obliqua</i>	WR	BIT	22.01±0.73	53.79
<i>Eucalyptus salmonophloia</i>	GWW	BIT	18.88±0.09	87.56
<i>Eucalyptus salubris</i>	GWW	BIT	18.02±0.19	96.82
<i>Eucalyptus socialis</i>	CM	BIT	19.94±0.18	76.17
<i>Eucalyptus tereticornis</i>	CP	BIT	21.27±0.26	61.85
<i>Eucalyptus tetradonta</i>	LF	BIT	19.82±0.34	77.39
<i>Eucalyptus transcontinentalis</i>	GWW	BIT	19.75±0.31	78.24
<i>Eucryphia lucida</i>	WR	BIT	22.07±0.06	53.16
<i>Ficus leptoclada</i>	RC	BIT	21.53±0.49	59.01
<i>Ficus variegata</i>	CT	BIT	21.85±0.29	55.60
<i>Flindersia bourjotiana</i>	RC	BIT	23.8±0.43	34.54
<i>Gillbeea adenopetala</i>	RC	BIT	24.41±0.26	27.94
<i>Gillbeea whypallana</i>	CT	BIT	23.22±0.42	40.79
<i>Leptospermum lanigerum</i>	WR	BIT	21.61±0.39	58.15
<i>Litsea leefeana</i>	RC	BIT	23.9±0.48	33.38
<i>Melaleuca squarrosa</i>	WR	BIT	21.94±0.25	54.60
<i>Myristica globosa</i>	CT	BIT	24.04±0.53	31.91
<i>Notelaea ligustrina</i>	WR	BIT	21.2±0.51	62.52
<i>Nothofagus cunninghamii</i>	WR	BIT	21.06±0.59	64.02
<i>Phyllocladus aspleniifolius</i>	WR	S	18.48±0.68	91.93
<i>Pittosporum bicolor</i>	WR	BIT	19.99±0.67	75.63
<i>Polyscias elegans</i>	RC	BIT	22.96±0.6	43.60
<i>Pomaderris apetala</i>	WR	S	23.55±0.44	37.21
<i>Prunus turneriana</i>	RC	BIT	25.83±0.74	12.59
<i>Rockinghamia angustifolia</i>	CT	BIT	22.69±0.35	46.49
<i>Synima cordierorum</i>	CT	BIT	22.8±0.39	45.33
<i>Syzygium johnsonii</i>	RC	BIT	25.7±0.07	13.99

Species	Site Code ¹	PFT	$\Delta^{13}\text{C}$ (‰)	Calculated WUE _i
<i>Syzygium sayeri</i>	CT	BIT	23.01±0.46	43.04
<i>Tasmannia lanceolata</i>	WR	S	21.8±0.26	56.03
<i>Xanthophyllum octandrum</i>	CT	BIT	22.64±0.6	47.08

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7 ¹ AMU = Alice Mulga; WR = Warra tall eucalypt; CT = Cape Tribulation; RC = Robson's Creek; GWW =
8 Great Western Woodlands; CM = Calperum Mallee; LF = Litchfield forest; CP = Cumberland Plain.

9 **Table S2: List of climate variables used in climate analysis**

WorldClim Code	Variables
BIO ₁	Mean Annual Temperature
BIO ₂	Mean Diurnal Range
BIO ₃	Isothermality
BIO ₄	Temperature Seasonality
BIO ₅	Max Temperature of Warmest Month
BIO ₆	Min Temperature of Coldest Month
BIO ₇	Temperature Annual Range
BIO ₈	Mean Temperature of Wettest Quarter
BIO ₉	Mean Temperature of Driest Quarter
BIO ₁₀	Mean Temperature of Warmest Quarter
BIO ₁₁	Mean Temperature of Coldest Quarter
BIO ₁₂	Mean Annual Precipitation
BIO ₁₃	Precipitation of Wettest Month
BIO ₁₄	Precipitation of Driest Month
BIO ₁₅	Precipitation Seasonality
BIO ₁₆	Precipitation of Wettest Quarter
BIO ₁₇	Precipitation of Driest Quarter
BIO ₁₈	Precipitation of Warmest Quarter
BIO ₁₉	Precipitation of Coldest Quarter

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17 **Table S3: Relationships of leaf traits with Mean Annual Precipitation and Moisture Index**

Traits ¹	Correlated with ln MAP						Correlated with MI					
	Dry-season			Wet-season			Dry-season			Wet-season		
	<i>Adj</i> <i>r</i> ²	<i>slope</i>	<i>p</i>	<i>Adj</i> <i>r</i> ²	<i>slope</i>	<i>p</i>	<i>Adj</i> <i>r</i> ²	<i>slope</i>	<i>p</i>	<i>Adj</i> <i>r</i> ²	<i>slope</i>	<i>p</i>
A ₄₀₀	0.224	+	<0.0001	NS		> 0.05	0.071	+	< 0.05	NS		> 0.05
ln E ₄₀₀	0.140	+	<0.001	0.142	+	< 0.01	0.04	+	> 0.05	NS		> 0.05
ln vpdL ₄₀₀	0.281	-	<0.0001	NS		> 0.05	0.119	-	<0.0001	NS		> 0.05
sqrt g _{s400}	0.414	+	<0.0001	0.119	+	< 0.01	0.142	+	<0.01	NS		> 0.05
ln WUE _i	0.481	-	<0.0001	0.420	-	<0.0001	0.206	-	<0.0001	0.357	-	<0.0001
C _i /C _a	0.332	+	<0.0001	0.337	+	<0.0001	0.192	+	<0.0001	0.380	+	<0.0001
C _{i400}	0.352	+	<0.0001	0.439	+	<0.0001	0.199	+	<0.0001	0.443	+	<0.0001
LDMC	0.349	-	<0.0001	0.331	-	<0.0001	0.277	-	<0.0001	0.366	-	<0.0001
ln LMA	0.569	-	<0.0001	0.534	-	<0.0001	0.412	-	<0.0001	0.504	-	<0.0001
ln FMA	0.532	-	<0.0001	0.516	-	<0.0001	0.369	-	<0.0001	0.452	-	<0.0001
ln Leaf N _{mass}	0.192	+	<0.001	0.264	+	<0.001	NS		> 0.05	0.302	+	<0.0001
ln Leaf P _{mass}	0.259	+	<0.0001	NS		> 0.05	NS		> 0.05	NS		> 0.05
ln Ratio N/P	0.102	-	<0.01	0.167	+	< 0.01	NS		> 0.05	0.09	+	< 0.05
ln Leaf N _{area}	0.178	-	<0.0001	0.269	-	<0.001	0.363	-	<0.0001	0.204	-	<0.01
ln Leaf P _{area}	N/S		> 0.05	0.399	-	<0.0001	0.228	-	<0.001	0.286	-	<0.0001
A ₄₀₀ ·N	0.403	+	<0.0001	0.162	+	<0.01	0.396	+	<0.0001	NS		> 0.05
ln A ₄₀₀ ·P	0.106	+	< 0.01	0.272	+	<0.001	0.334	+	<0.001	0.119	+	<0.01

NS = Non-significant

¹E = transpiration rate; VPD = vapour pressure deficit; g_s = stomatal conductance; WUE_i = intrinsic water-use-efficiency; C_i/C_a = ratio of internal to external concentrations of CO₂; LDMC = leaf dry matter content; LMA = leaf mass to area ratio; ; FMA = foliar mass per unit area; N_{mass} = foliar N content expressed per unit dry weight; P_{mass} = foliar P content per unit mass; N_{area} = foliar N content expressed per unit leaf area; P_{area} = foliar P content per unit leaf area;

Table S4: Variation in $\Delta^{13}\text{C}$ and WUE_i by biome

Mean and s.e of wet and dry season $\Delta^{13}\text{C}$ and WUE_i . Means followed by different letters across sites in each season are significantly different (Tukey HSD, confidence level of 0.05).

Biome ¹	$\Delta^{13}\text{C}$ (‰)		WUE_i (‰)	
	Dry-season	Wet-season	Dry-season	Wet-season
SW	19.75±0.49c	19.15±0.32a	78.14±5.26a	84.60±4.30a
STS	19.82±0.25c	20.15±0.40a	77.35±2.74a	73.85±3.45a
TW	20.16±0.25c	20.18±0.28a	73.84±4.10a	73.57±3.03a
HTS	20.77±0.38b,c	-	67.21±2.84a,b	-
TWF	21.54±0.55b,c	23.51±0.56b	68.80±5.91a,b	37.68±6.12a,b
LTR	22.98±0.46a,b	23.02±0.39b	43.25±4.93a,b	42.86±4.17a,b
UTR	24.05±0.76a	24.31±0.52b	31.74±8.16a	29.04±5.62b

¹ SW = semi-arid woodland; STS = semi-arid tropical savanna; TW = tropical woodland; HTS = high rainfall tropical savanna; TWF = temperate wet forest; LTR = lowland tropical rainforest; UTS = upland tropical rainforest

63 **Brief site descriptions of the seven Supersites (8 nodes)**

64 **Calperum Mallee (CM)**

65 The Calperum Mallee SuperSite is in the mallee semi-arid ecosystem located approximately 25 km
66 north of Renmark in South Australia. The landscape is an extensive plain with undulating mallee
67 woodland and riverine vegetation that fringes the River Murray and its anabranches. The vegetation
68 is dominated by upper storey Eucalypt trees of four species (*Eucalyptus dumosa*, *Eucalyptus*
69 *incrassata*, *Eucalyptus oleosa* and *Eucalyptus socialis*) (Meyer *et al.*, 2015).

70 **Great Western Woodlands (GWW)**

71 The Great Western Woodlands located in south-west Western Australia is the largest remaining
72 intact semi-arid temperate woodland in the world. The vegetation comprises a 16-million hectare
73 mosaic of mallee, scrub–heath and woodland and is locally determined by edaphic factors and
74 influenced by historic disturbances (Gosper *et al.*, 2013). Mean annual rainfall is ~250 mm with the
75 highest-mean rainfall months in winter. *Eucalyptus salubris* constructs the dominant crown layer in
76 association with other *Eucalyptus* species (*E. salmonophloia*, *E. longicornis* and *E. moderata*) (Gosper
77 *et al.*, 2013).

78 **Alice Mulga (AMU)**

79 The semi-arid Alice Mulga SuperSite is located approximately 200 km north of Alice Springs, in the
80 Northern Territory of Australia. The climate is characterized as having hot summers and warm
81 winters. Mean annual rainfall is ~300 mm and is highly seasonal, mostly occurring in large rainfall
82 events during summer. Vegetation is dominated by Mulga (*Acacia aneura* and related species)
83 woodlands, occasionally with large areas of spinifex under sparse woodland of *Corymbia* and other
84 *Acacia* species (Cleverly *et al.*, 2016).

85 **Cumberland Plain (CP)**

86 The Cumberland Plain is a sclerophyll *Eucalyptus* woodland west of Richmond in New South Wales.
87 The soil is characterized by nutrient-poor alluvium from sandstone and shale bedrock in the Blue
88 Mountains deposited by the Nepean River. Despite being nutrient poor, this SuperSite supports high
89 regional biodiversity and endemic biota and is dominated by *Eucalyptus fibrosa*, *E. moluccana* and *E.*
90 *tereticornis* in the overstorey. Mean annual precipitation of this site is 900 mm (Table 1).

Warra Tall Eucalypt (WR)

The Warra Tall Eucalypt SuperSite is a cool, wet temperate forest located in Tasmania. Vegetation is dominated by tall *Eucalyptus obliqua* occurring in a full range of successional stages from young regrowth forests to old-growth mixed forests (Hickey *et al.*, 1999). Mean annual temperature at this site is the lowest (~10°C), with a mean annual precipitation of 1474 mm (Table 1).

Litchfield Savanna Forest (LF)

The Litchfield Savanna SuperSite is a ~1.5 km² tropical savanna 70 km south of Darwin in northern Australia. This site is representative of the dominant ecosystem of that region. Climate of this site is typical of northern Australia with extremely seasonal and high rainfall and approximately 56% of this site is burnt annually (Murphy *et al.*, 2010). However, in this study, data collected from Howard Springs (approximately 65 km north of Litchfield SuperSite; (Cernusak *et al.*, 2011) have been used as a representative of this particular SuperSite. This approach is justified because both of these sites had very similar vegetation and climate conditions as well as frequency of occurrence of fire. The stand structure in these two sites are sufficiently similar as to not shift physiological properties at the leaf-scale given the species occurred in both sites largely overlap (Bowman *et al.*, 2001; Hutley and Beringer, 2010; Murphy *et al.*, 2010).

FNQ Rainforest (RC and CT)

The Far North Queensland Rainforest SuperSite is located in a tropical wet forest ~140 km north of Cairns in Far North Queensland. This SuperSite is structurally divided into two transects – a) the lowland rainforest based in the Daintree rainforest near Cape Tribulation (CT; MAT = 25.2 °C, Ozflux site average MAP = 5700 mm) and b) the upland rainforest based around Robson Creek (RC; MAT = 21 °C, MAP = 2140 mm). Precipitation is highly seasonal with most occurring during summer (Weerasinghe *et al.*, 2014). FNQ supports 10% of Australian flora despite of occurring in only 0.2% its landmass. Consequently a substantial number of the species in this study comes from this SuperSite. Data from two nodes of this SuperSite, i.e., Cape Tribulation and Robson Creek were collected and analysed independently in this study because of significantly different environmental clines (altitude, MAT and MAP) that exists in these two nodes of the Far North Queensland sites.

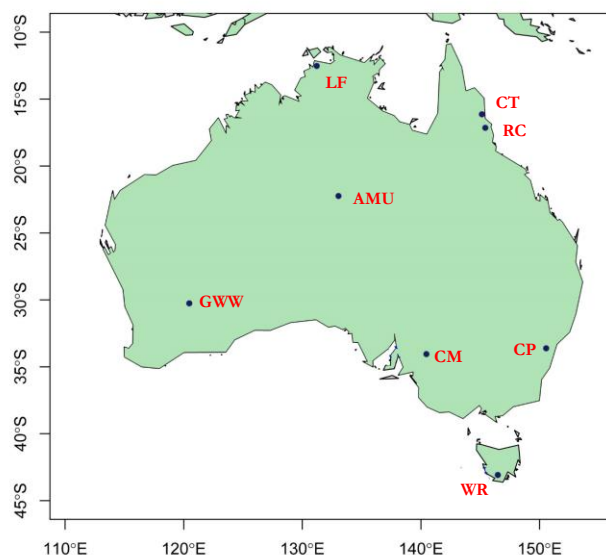


Figure S1: Location of SuperSites (represented by black dots).

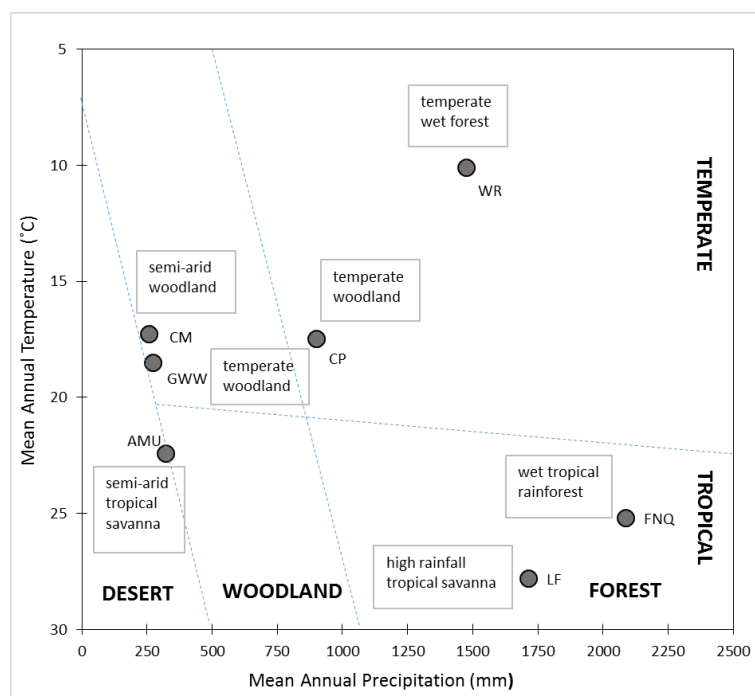


Figure S2: Mean annual temperature, mean annual precipitation, and biomes of the study sites.
Each SuperSite is plotted in the Whittaker Biome Diagram (Whittaker, 1975) using the MAT and MAP observations generated for each site from the WorldClim data.

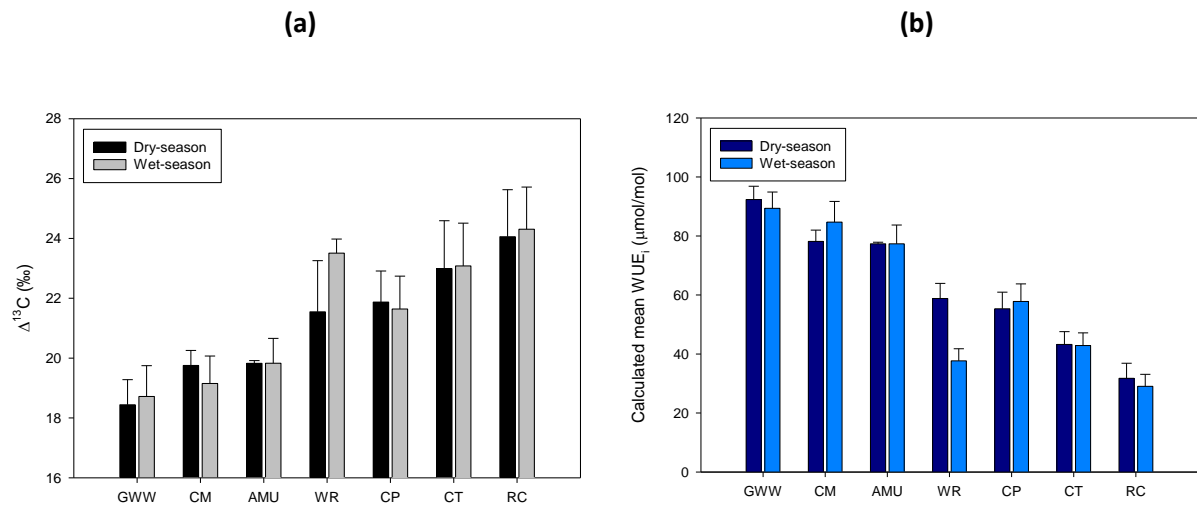


Figure S3: Site mean values of (a) $\Delta^{13}\text{C}$ and (b) WUE_i
 Dark and light bars represent mean of dry and wet-season respectively and the error bars represent standard errors.

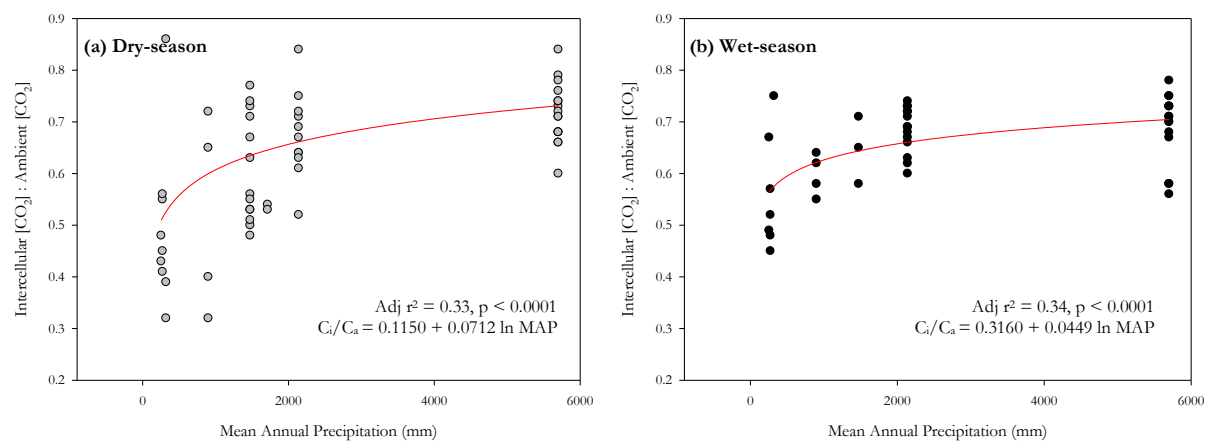


Figure S4: Ratio between intercellular and ambient $[\text{CO}_2]$ (C_i/C_a) for both seasons plotted as functions of mean annual precipitation (MAP).

Left and right panels are plotted from dry- and wet-season data respectively. Statistically significant correlations with MAP are plotted with red regression lines.

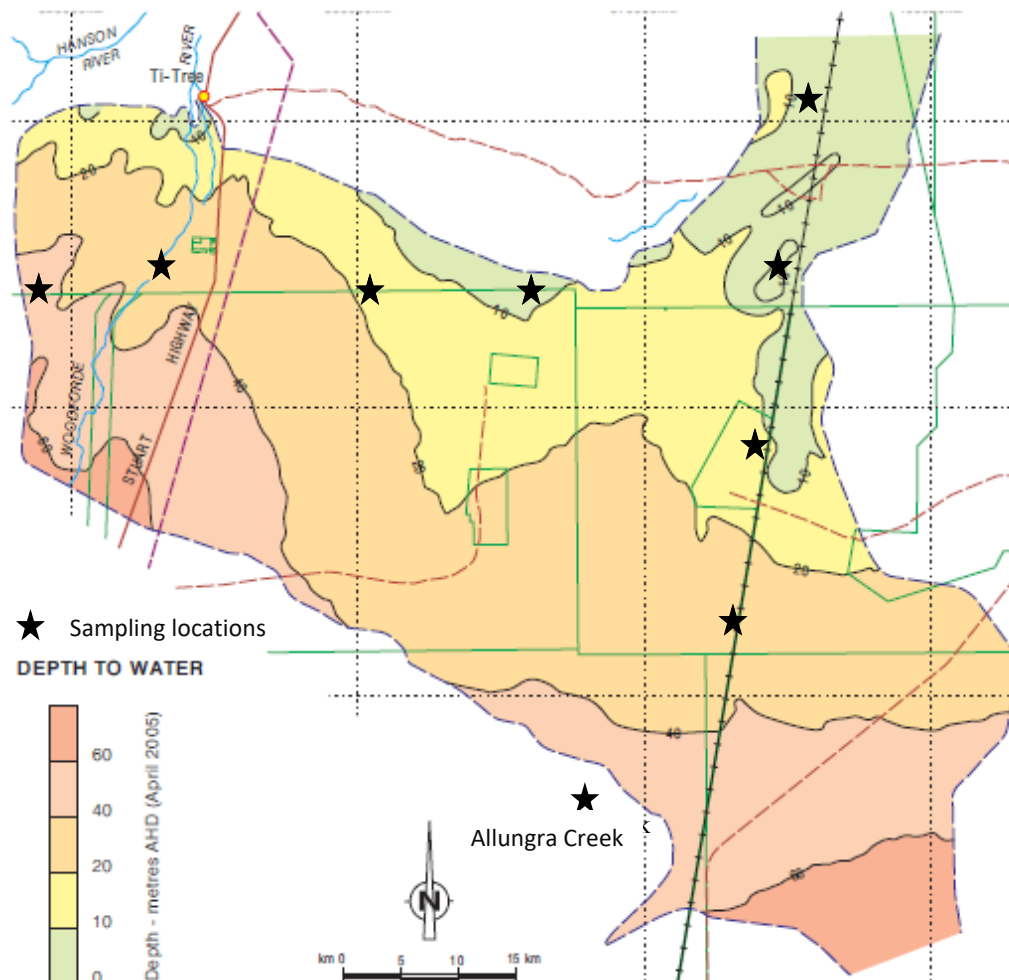


Figure S5: Map of the Northern Territory (NT) of Australia and the study area within the NT and the Ti Tree basin. Green lines represent property boundaries. AHD = Australian Height Datum