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Ant thermal tolerances under climate, land cover and land use change

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Thermal stress is a key issue for species dominant within ecosystems especially those that carry out key ecosystem service roles. When assessing the impacts of climate change it is critical to assess its biotic impacts relative to other anthropogenic changes to landscapes including the reduction of native vegetation cover, landscape fragmentation and changes in land use intensity. Here we integrate the observed phenotypic plasticity of the dominant and ubiquitous meat ant *Iridomyrmex purpureus* in critical thermal limits across altitudinal, land cover and land use gradients to: (i) predict the adaptive capacity of a key terrestrial ecosystem service provider to changes in climate, land cover and land use, and (ii) assess the ability of multiple use landscapes to confer maximum resilience to terrestrial biodiversity in the face of a changing climate. The research was carried out along a 270km aridity gradient spanning 840m in altitude in northern New South Wales, Australia. When we assessed critical thermal maximum temperatures (CT_{max}) of meat ants in relation to the environmental variables, and within the model we had critical thermal minimums of meat ants (CT_{min}) as a random slope and as a fixed effect we detected a negative aridity effect on CT_{max} , a negative effect of land use intensity, and no overall correlation between CT_{max} and CT_{min} . We also found a negative relationship with warming tolerance of *I. purpureus* and landscape aridity. In conclusion, we expect to see a reduction in the physiological resilience of *I. purpureus* as land use intensity increases and as the climate becomes more arid. Meat ants are key ecosystem engineers and as they are put under more stress, wider ecological implications may occur if populations decline or disappear.

1 **Ant thermal tolerances under climate, land cover and land use change**

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8

9 **Abstract**

10 Thermal stress is a key issue for species dominant within ecosystems especially those that
11 carry out key ecosystem service roles. When assessing the impacts of climate change it is
12 critical to assess its biotic impacts relative to other anthropogenic changes to landscapes
13 including the reduction of native vegetation cover, landscape fragmentation and changes in
14 land use intensity. Here we integrate the observed phenotypic plasticity of the dominant and
15 ubiquitous meat ant *Iridomyrmex purpureus* in critical thermal limits across altitudinal, land
16 cover and land use gradients to: (i) predict the adaptive capacity of a key terrestrial ecosystem
17 service provider to changes in climate, land cover and land use, and (ii) assess the ability of
18 multiple use landscapes to confer maximum resilience to terrestrial biodiversity in the face of a
19 changing climate. The research was carried out along a 270km aridity gradient spanning 840m
20 in altitude in northern New South Wales, Australia. When we assessed critical thermal
21 maximum temperatures (CT_{max}) of meat ants in relation to the environmental variables, and
22 within the model we had critical thermal minimums of meat ants (CT_{min}) as a random slope and
23 as a fixed effect we detected a negative aridity effect on CT_{max} , a negative effect of land use
24 intensity, and no overall correlation between CT_{max} and CT_{min} . We also found a negative
25 relationship with warming tolerance of *I. purpureus* and landscape aridity. In conclusion, we
26 expect to see a reduction in the physiological resilience of *I. purpureus* as land use intensity
27 increases and as the climate becomes more arid. Meat ants are key ecosystem engineers and as

28 they are put under more stress, wider ecological implications may occur if populations decline
29 or disappear.

30 **Introduction**

31 Assessing the performance and physiological responses of ectotherms is critical to
32 understanding biotic responses to climate change (Andrew & Terblanche 2013), particularly the
33 effects of exposure to thermal stress and temperature extremes (Vasseur et al. 2014) on key
34 ecosystem service providers. Biochemical and physiological reactions are mediated by
35 temperature and thermal stress can negatively influence development, growth, metabolism,
36 movement and reproduction, leading to changes in community and ecosystem level processes
37 (Dell et al. 2011; Grigaltchik et al. 2012). Thermal performance curves identify how the
38 performance or fitness of an ectotherm is influenced by body temperature (Sinclair et al. 2016),
39 a key response to thermal stress identified by these curves are critical thermal limits: the
40 functional endpoint that identify upper and lower limits of temperatures that insects can
41 tolerate from which they are unable to escape (Lighton & Turner 2004).

42 Thermal stress is a key issue for all taxa including those that provide key ecosystem services
43 and are dominant within ecosystems (Andrew 2013; Andrew et al. 2016; Andrew et al. 2013a;
44 Mooney et al. 2009). Through many terrestrial ecosystems worldwide, ants provide key
45 ecosystem services and mediate key ecosystem processes (Del Toro et al. 2012; Hölldobler &
46 Wilson 1990). The resilience of these ecosystem service providers to thermal stress may change
47 among populations as they are exposed to different environmental conditions, particularly in a
48 more variable climate (Greenslade 1976). This phenotypic (or physiological) plasticity is a core
49 driver of adaptive responses to climatic variation (Kingsolver & Huey 1998).

50 It is especially critical to take into account the impacts of climate change relative to other
51 anthropogenic changes to landscapes including the reduction of native vegetation cover,
52 landscape fragmentation and changes in land use intensity (Oliver & Morecroft 2014; Sala et al.
53 2000). Assessing the synergistic effects of changes in land cover, land use and climate are
54 critical to enable decision makers to make better determinations in regards to the management

55 and conservation of biodiversity, ecosystem and environmental services both now and into the
56 future (Mawdsley et al. 2009). Previously we assessed how adapting landscapes may improve
57 insect biodiversity conservation via a study of the additive and synergistic effects of climate
58 with land cover and land use change (Oliver et al. 2016). From the main-effects models
59 developed, it was found that a greater amount of woody plant canopy cover increases ant
60 richness (species and genus) and diversity; whereas a higher amount of land cultivation,
61 grazing, exotic plant groundcover and bare ground reduced species richness. At sites with
62 warmer and drier climates (i.e. a higher aridity index), native plant canopy cover had greatest
63 benefit, and exotic plant cover had the most negative effects, on ant species richness (Oliver et
64 al. 2016). From this, we predict that the effects of landscape change on diversity may also affect
65 the thermal physiology of insect populations.

66 Changes in a dominant and widespread ectotherm's critical thermal limits across climatic,
67 land use and land cover gradients may occur (Angilletta et al. 2007): leading to changes in
68 community structure and the provision of ecological services (Traill et al. 2010). Exposure to
69 different microclimates may influence ectotherm physiology in more unpredictable ways than
70 just exposure to warmer temperatures individually. Microclimates that ants are exposed to (e.g.
71 Andrew et al. 2013a; Hemmings & Andrew 2017) may change substantially across surfaces
72 within different habitat spaces: such as those with substantive bare ground, a high grazing
73 intensity, exotic plant species cover, and woody ground cover.

74 Here we focus on meat ants (*Iridomyrmex purpureus* (Smith, F., 1858)) as they are a
75 dominant and ubiquitous part of the landscape (Andersen 2000; Greaves 1971; Greenslade
76 1976). *Iridomyrmex purpureus* can have a substantive impact on the availability of resources
77 and the use of these resources by other species in different landscapes (Gibb 2005).
78 *Iridomyrmex purpureus* are also excellent at resource exploitation and interference competition
79 to enable them to dominate and control resources quickly (Gibb & Hochuli 2004). They can also
80 maximise their foraging times by displaying opportunistic thermal responses and adjusting
81 foraging behaviour to deal with high trail temperatures (Andrew et al. 2013a).

82 Warming tolerance defines how much warming an ectotherm can tolerate before lethal
83 levels are attained (Deutsch et al. 2008): it is calculated by taking the difference between the
84 upper critical thermal limit and the habitat ambient temperature. These values can change
85 substantially based on the method from which habitat temperatures are derived. For example,
86 Andrew *et al.* (2013a) found *I. purpureus*' warming tolerance at a site in temperate Australia
87 (Armidale, New South Wales (NSW)) to be relatively high (25.8°C) when habitat temperatures
88 were based on closest weather station annual averages, but warming tolerance reduced when
89 closest weather station summer average temperatures and then microclimate summer average
90 temperatures were used (19.52°C and 19.12°C respectively). Warming tolerance decreased
91 substantially (to 7.81°C) when microclimate temperatures based on summer temperatures
92 between 10am and 4pm (when ants are most surface active) were used.

93 In this study we integrate observed ant phenotypic plasticity in critical thermal limits across
94 altitudinal, land cover and land use gradients to: (i) predict the adaptive capacity of terrestrial
95 invertebrate biodiversity to changes in climate, land cover and land use, and (ii) assess the
96 ability of multiple use landscapes to confer maximum resilience to terrestrial biodiversity in the
97 face of a changing climate.

98 The following questions were addressed:

99 What were the critical thermal limits (CT_{max} and CT_{min}) for *I. purpureus* across sites
100 representing the main climatic, vegetation, and land-use regimes?

101 What were the key environmental drivers (climatic, vegetation, and land-use regimes)
102 influencing thermal limits?

103 What is the relationship between *I. purpureus*' warming tolerance and aridity along the
104 environmental gradient?

105 **Methods**

106 *Site selection*

107 The study was carried out along a 270km aridity gradient spanning 840m in altitude in
108 northern NSW, Australia (Table 1). The area has some of the most fertile soils in Australia, with
109 much of the farming practices dominated by livestock grazing on modified pastures and native
110 vegetation, and dryland and irrigated cropping (BRS 2009). Native remnant vegetation is
111 dominated by semi-arid woodlands at lower altitudes through to grassy woodlands and dry
112 sclerophyll forest at higher altitudes (Keith 2004). Eleven sites were chosen to represent a
113 range of climatic, land-use and native woody vegetation cover along this gradient (Table 1)
114 covering the total number of sites (87) that were used to assess ant species diversity (Oliver et
115 al. 2016). Sites were chosen to maximise the range in climate (Aridity: based on rainfall and
116 evaporation collected from modelled climate data from ANUCLIM 6.1 (Xu & Hutchinson 2011)
117 over three time periods: 3 months, 12 months and 36 months), land cover (total native woody
118 cover (Canopy) and bare ground), land use (intensity of use: Land Use Intensity - LUI, and exotic
119 groundcover) and soil pH and clay content. Land use intensity is a semi-quantitative index
120 based on cultivation and grazing severity and age: so more intensively managed sites have
121 higher values (ranging between 0 and 12). More information on how these variables were
122 calculated and justified can be found in Oliver *et al.* (2016).

123 We used the ant dataset collected from Oliver *et al.* (2016) to identify a common and
124 widespread species to assess for critical thermal limits. *Iridomyrmex purpureus* was chosen for
125 physiological tolerance comparisons as it was the most abundant species at each site. A
126 minimum of 15 individual ants were collected from each site between April and May 2014, and
127 then held at 25°C for two hours to avoid effects of time of day of capture differences along the
128 gradient. Previous work on *I. purpureus* found no effect of time of day of capture/ nest
129 temperature on thermal tolerances (determined via thermolimit respirometry) from a single
130 site (Andrew et al. 2016).

131 *Critical thermal maximum and minimum assessments (CT_{max} and CT_{min})*

132 CT_{max} measurements were carried out in a Grant R4 waterbath with a GP200 heater using
133 distilled water. Ten individuals from each site were each put into a single 50ml vial for testing,
134 and a reading for each ant was taken. Waterbath temperature was initialised for 10 minutes at

135 25°C and then ramped at 0.25°C/minute until CT_{max} was reached. CT_{max} was identified when an
136 individual ant could not perform coordinated motor functions in the vial to right itself after
137 being turned onto its side (Andrew et al. 2013a). CT_{max} could go up to 55°C (equivalent to 120
138 minutes/individual – 30°C temperature change at 0.25°C/min). Ramping at 0.25°C is considered
139 the most ‘standard’ temperature ramping rate, at which the body temperature of ants is in
140 equilibrium with their surroundings (Andrew et al. 2013a; Chown et al. 2009; Lighton & Turner
141 2004; Nguyen et al. 2014; Terblanche et al. 2007). CT_{min} was carried out in a similar fashion to
142 CT_{max} using 1:1 distilled water/glycol mix. Waterbath temperature was initialised for 10 minutes
143 at 5°C and then decreased at 0.25°C/minute until CT_{min} was reached. CT_{min} was identified when
144 an individual ant could not perform coordinated motor functions in a 50ml vial to right itself
145 after being turned onto its side (Andrew et al. 2013a). CT_{min} could go down to -15°C (equivalent
146 to 80 minutes/species – 20°C temperature change at 0.25°C/min). To measure temperatures
147 that ants were exposed to within each vial, a Type-T thermocouple was placed within another
148 50ml vial that was plunged with the ants and connected to a temperature datalogger (Testo
149 175 T3) with data logged as waterbath temperatures were ramped: the Testo temperature was
150 used to identify ant $CT_{max/min}$.

151 *Model fitting*

152 We used *R* (R Core Team 2017) and the R package *lme4* (Bates et al. 2015) to perform a
153 linear mixed effects analysis of the relationship between CT_{max} as a response variable against
154 the environmental variables of aridity, LUI (converted to a proportion), soil clay content, exotic
155 plant ground cover, and total native woody cover (Canopy) designated as fixed effects. We
156 explored singular interaction effects of Aridity:LUI, Canopy:LUI, and Clay:LUI in some models as
157 well as the impact of dropping main effect variables. With this framework we considered
158 random intercept models by site only, and by both site and CT_{min} (individually). We also
159 considered a random intercept, random slope model with CT_{min} within Site as the random
160 effect. We repeated this model selection process with CT_{min} as the response variable and CT_{max}
161 as the predictor variable where appropriate. All variables were centred and scaled. Models
162 were initially fit with REML and then refitted with ML for comparison in Likelihood ratio tests.

163 Minimum AIC values and p-values of less than 0.05 were used to aid model selection. Visual
164 inspection of residual plots of the preferred models were used to assess obvious deviations
165 from homoscedasticity or normality. Visualization of random effects were undertaken using R
166 package *sjPlot* (Lüdecke 2017). Standard errors and confidence intervals for predicted values of
167 preferred models were undertaken using parametric bootstrapping (n = 1000) within R package
168 *bootpredictlme4* (Duursma 2017) and visualized within R package *visreg* (Breheny & Burchett
169 2017).

170 *Warming Tolerance*

171 Warming tolerance was calculated using the equation of Deutsch *et al.* (2008) and Diamond
172 *et al.* (2012): $CT_{max} - T_{hab}$. The T_{hab} calculation may include different calculations (e.g. annual
173 average; summer average; microclimate summer average; and microclimate summer 10am-
174 4pm summer average) which are ecologically relevant and to identify the most appropriate to
175 assess ectotherm stress (e. g. Andrew *et al.* 2013a). For T_{hab} here, we did not have access to
176 microclimate data, so we modelled site location data using ANUCLIM V6.1 (Xu & Hutchinson
177 2011) from the closest weather stations based on 3 month summer average 2009, 12 month
178 average for 2009 and 36 month (2007-2009) average day temperatures. These weather data
179 were used, as the data were generated for all sites at the time of sampling ant species richness
180 in Oliver *et al.* (2016).

181 **Results**

182 Critical thermal maxima of individual ants ranged between 41.5°C and 56.1°C, and CT_{min}
183 between 0.3°C and 7.1°C in this study. There was no consistent relationship between CT_{min} and
184 CT_{max} across the 11 sites sampled (Figure 1), suggesting no causal relationship between the two
185 end points.

186 The preferred model proposed for explaining meat ants CT_{max} across the landscape is:

187 $CT_{max} \sim LUI + Canopy + Exotic + Clay + Aridity + (CT_{min} | SITE_ID)$

188 The fixed effects for this model are shown in Table 2. The overall random effects for the
189 model above are (in terms of variance): Site: 1.0448; $CT_{\min} | \text{Site}$: 0.1937; and Residuals: 1.4886.
190 As shown in Figure 2, Sites are an important source of variation (much more so than CT_{\min}
191 although the inclusion of this was still significant as per the model selection process). However,
192 there is still additional (unaccounted for) variation in the residuals. For the variables of LUI and
193 Clay, there were significant relationships with CT_{\max} (Figure 3). As LUI increases, CT_{\max}
194 decreases; whereas clay content was positively correlated with CT_{\max} .

195 For explaining mean ants CT_{\min} across the landscape a similar model is proposed as that for
196 CT_{\max} :

$$197 \quad CT_{\min} \sim \text{LUI} + \text{Canopy} + \text{Exotic} + \text{Clay} + \text{Aridity} + (CT_{\max} | \text{SITE_ID})$$

198 The fixed effects for this model are shown in Table 3. The overall random effects for the
199 model above are (in terms of variance): Site: 1.3011; $CT_{\max} | \text{Site}$: 0.3484; Residuals: 1.3769. As
200 with CT_{\max} , the sites also exhibit a high amount of variation (Figure 2). The prediction intervals
201 for CT_{\min} also show similar results as those for CT_{\max} , (Figure 3c and d) however the
202 relationships are weaker for both LUI and Clay content.

203 We found a negative relationship between the warming tolerances of *I. purpureus* and
204 landscape aridity (Figure 4). This relationship was consistent among all measures of mean
205 temperatures (no significant difference in Test for Common Slope across Groups: Test Statistic =
206 1.488, $p = 0.482$). There was a significant difference in the slope elevation of warming tolerance
207 between the three month and thirty six month mean temperature calculations (Figure 4; d.f. =
208 2, WALD = 95.299, $p < 0.0001$).

209 Discussion

210 The phenotypic plasticity in critical thermal limits and physiological responses of insects to a
211 changing climate is crucial for understanding how individuals and populations will respond to
212 changes in their local environment (Andrew et al. 2013b; Andrew & Terblanche 2013). These
213 responses are becoming a key area of research interest (Andrew et al. 2013a). The assessment

214 of common species responses to a changing climate needs to be thoroughly assessed, as
215 changes in these taxa's population structure can have large implications for the ecosystems in
216 which they provide key services (Andrew 2013; Gaston 2011; Inger et al. 2014). In addition, as
217 landscapes become more fragmented and anthropogenic, common and dominant species
218 responses to changes may also be limited. Here, critical thermal maxima and minima were
219 determined for ants that encompassed an extensive distribution along an environmental
220 gradient.

221 There was no strong pattern in CT_{max} and CT_{min} associated with the environmental variables
222 tested. The results of the CT_{max} measurements indicates there is a high variation of CT_{max} across
223 sites, this may be due to the ants being field fresh and so their previous exposure to a variety of
224 stresses may influence their thermal capabilities. However, this is also important, as it indicates
225 that no one individual stress dominates the thermal abilities of *I. purpureus* workers on site.

226 Critical thermal maxima of individual ants ranged between 41.5°C and 56.1°C, and CT_{min}
227 between 0.3°C and 7.1°C in this study. This is a very wide range of readings for CT_{max} , and could
228 be due to age, nutritional status, stress or prior heat exposure that the ants may have been
229 exposed to (Nyamukondiwa & Terblanche 2009; Sørensen et al. 2001). Upper thermal limits are
230 thought to be less plastic compared to lower limits, however it is known that environmental
231 exposure does influence these limits (Hoffmann et al. 2013), and this is seen with the
232 relationships with both LUI and Clay in this study. Here we measured CT_{max} and CT_{min} by
233 observing an individual ant's ability to right itself while temperatures were increasing at 0.25°C
234 min⁻¹. The calculation of critical thermal limits using ant righting behaviour may be more
235 variable than using physiological critical limits such as upper lethal temperatures where ants
236 are exposed to static temperatures for two hours (Andrew et al. 2013a) or thermolimit
237 respirometry where CT_{max} is derived from metabolic measurements using flow-through CO₂-
238 based respirometry and optical detection, when temperatures are ramped at a consistent rate
239 (Andrew et al. 2016; Lighton & Turner 2004). As an alternative measure of CT_{max} thermolimit
240 respirometry (Lighton & Turner 2004) may be more robust, as the method explicitly measures
241 the ceasing of metabolism (release of carbon dioxide) of the ant; but it is also a different

242 measure of CT_{max} , as there is no ability for the ants to recover from heat exposure in
243 thermolimit respirometry.

244 When the fitted models were used to assess critical thermal limits, it is clear that site specific
245 differences strongly influenced the results found. However, land use and soil clay content also
246 played a significant role in influencing ant physiological end-points. This suggests that ant
247 populations that were exposed to higher levels of habitat modification (via land use intensity)
248 showed lower climatic resilience relative to less disturbed habitats. However, there is still
249 additional unaccounted for variation in the residuals which suggests that there may be other
250 variables (unmeasured) that may have an effect on the meat ants' climatic resilience.

251 For ants, much of the research on local effects of habitat disturbance has been carried out
252 on changes in communities (Andersen & Majer 2004; Andrew et al. 2000; Bromham et al.
253 1999). Previous work carried out along the gradient (Oliver et al. 2016) used for this study
254 found clear evidence for landscape adaptation to maintain and restore species richness of ant
255 communities at the site level. For ant communities, higher woody native cover and shrub cover,
256 and lower exotic plant groundcover have a positive effect on ant species richness. Interestingly,
257 Land Use Intensity had no significant impact on the species richness within any of the ant
258 genera assessed across the gradient.

259 However, clay did have a positive influence on *Iridomyrmex* spp. diversity in Oliver et al.
260 (2016). Clay and clay-like substrate is an important component for ant nest development
261 (Monaenkova et al. 2015), and is critical for other insect taxa, such as termites in giving their
262 feeding galleries structural support to assist with load bearing (Oberst et al. 2016). As *I.*
263 *purpureus* nests are known to be located in the same location for over 70 + years (Greenslade
264 1975), substantive structural elements are required to keep it maintained during this time. The
265 amount of clay in a *I. purpureus* nest is representative of the surrounding non-nest soil
266 (Ettershank 1968). *Iridomyrmex purpureus* nests are also not found on quartz sand soils, even
267 when climatic factors are suitable, indicating that soil type can be a limiting distributional factor
268 (Greaves 1971). As clay plays a role in the distribution of the species, it also clearly plays a role in
269 the physiological breadth of individuals.

270 For the CT_{max} model fitting, there was one model with a lower AIC (in which the interaction
271 between LUI*Canopy) was included. This was a more complex model, and so was not deemed
272 the most appropriate to best explain the CT_{max} relationship. In addition, the current model is
273 simpler to interpret and also in line with the results for CT_{min} .

274 We calculated warming tolerance using three different measures of habitat temperature, all
275 generated based on location data using ANUCLIM. These all indicated, as expected, that an
276 increase in aridity reduces ant tolerance to warming. When the warming tolerance was
277 previously calculated for *I. purpureus* at a higher altitude (Armidale, NSW: 980 masl), similar
278 calculations were made: a warming tolerance of 19.5°C was calculated on weather station
279 summer average, and 25.8°C based on weather station annual average (Andrew et al. 2013a).
280 As Armidale is a more temperate site than those tested here, it would be at the lower scale of
281 the aridity index. Across the aridity index there is a 10°C difference in warming tolerance for *I.*
282 *purpureus*. With a prediction of global increases in air temperature of 2°C and 6°C over the 21st
283 Century, and in the region assessed there is an 80% probability of a 3°C warming and a 30%
284 probability of a 4°C warming with a likelihood of reduced annual rainfall of 3-5% (CSIRO-ABM
285 2012), aridity of the region assessed will only continue to increase.

286 Conclusion

287 From this study we have found that habitat type (e.g. soils) and land-use intensity are more
288 limiting factors on meat ant CT_{max} and CT_{min} than climatic factors (here we tested aridity). These
289 populations are key ecosystem engineers and as they are put under more stress, wider
290 ecological implications may occur if population abundances decline, as we expect to see a
291 reduction in the physiological resilience of *I. purpureus* as land use intensity increases.

292

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Table 1 (on next page)

Characteristics of the 11 sites used in this study (from a total of 87). See Oliver *et al.* (2016) for details on the full complement of sites.

- 1 Table 1. Characteristics of the 11 sites used in this study (from a total of 87). See Oliver *et al.* (2016) for details on the full complement of sites.

Site name	Aridity	Altitude	Land Use Intensity	Soil Clay	Total native woody cover	Exotic Ground Cover	pH	Lat	Long
Smokey Mountain (38)	0.401	891	2	5	23	50	4.585	-29.966	151.271
Furrocabad Station (44)	0.346	1047	3	28.8	30	69	5.25	-29.83	151.608
Furrocabad Station (45)	0.366	1008	8	28.8	97	68	5.64	-29.823	151.598
Delunga 52	0.617	338	0	37.5	76	9	6.16	-29.835	150.554
62	0.716	203	4	27.5	16	43	5.675	-29.379	149.797
63	0.715	204	5	30	66	2	5.855	-29.378	149.796
87C	0.732	163	6	57.5	1	83	6.425	-29.693	149.23
Towarra (96)	0.537	643	1	28.8	70	1	5.56	-30.125	150.76
Myall Creek (117)	0.583	457	1	65	68	18	6.465	-29.823	150.74
West Oaks (126C)	0.491	730	4	15	97	2	5.89	-29.359	151.429
West Oaks (127C)	0.508	683	5	53.8	1	10	5.695	-29.36	151.412

2

Table 2 (on next page)

Estimated fixed effects for the selected CT_{\max} model. Standard errors and 95% confidence intervals are also presented. All variables have been centred.

- 1 Table 2. Estimated fixed effects for the selected CT_{max} model. Standard errors and 95% confidence
- 2 intervals are also presented. All variables have been centred.

	Estimate	Std. Error	2.5 %	97.5 %
(Intercept)	46.04	0.30	45.52	46.54
Land Use Intensity (LUI)	-0.28	0.15	-0.48	-0.06
Total native woody cover (Canopy)	0.01	0.01	-0.014	0.02
Exotic groundcover	0.01	0.01	-0.01	0.03
Soil Clay Content	0.04	0.02	0.01	0.06
Aridity Index	2.82	2.54	-6.37	0.18

3

Table 3 (on next page)

Estimated fixed effects for the selected CT_{\min} model. Standard errors and 95% confidence intervals are also presented. All variables have been centred.

- 1 Table 3. Estimated fixed effects for the selected CT_{min} model. Standard errors and 95% confidence
- 2 intervals are also presented. All variables have been centred.

	Estimate	Std. Error	2.5%	97.5%
(Intercept)	3.7	0.37	2.85	4.28
Land Use Intensity	-0.091	0.18	-0.34	0.15
Total native woody cover (Canopy)	0.00	0.013	-0.03	0.02
Exotic Groundcover	0.004	0.016	-0.02	0.023
Soil Clay Content	0.017	0.025	-0.01	0.06
Aridity Index	0.29	3.3	-4.24	5.01

3

Figure 1(on next page)

Sites used in this study in northern New South Wales, Australia (see insert) with relative values for CT_{max} (A), Land Use Intensity (B), Aridity (C), and Clay (D) shown.

Maps generated using Map data © OpenStreetMap contributors. The size of the circle is indicative of the mean value of the given variable (i.e. the larger the circle, the higher the value, and the contrary). Image produced using the Leaflet package (version 1.1.0.9000, <http://rstudio.github.io/leaflet/>) within R statistical software (version 3.4.3). The R package OpenStreetMap is licensed under a GNU General Public License (GPL-2) (<https://cran.r-project.org/web/packages/OpenStreetMap/index.html>) and was used to extract map tiles from OpenStreetMap which is licensed on terms of the Open Database License, "ODbL" 1.0. (<http://wiki.osmfoundation.org/wiki/Licence>).

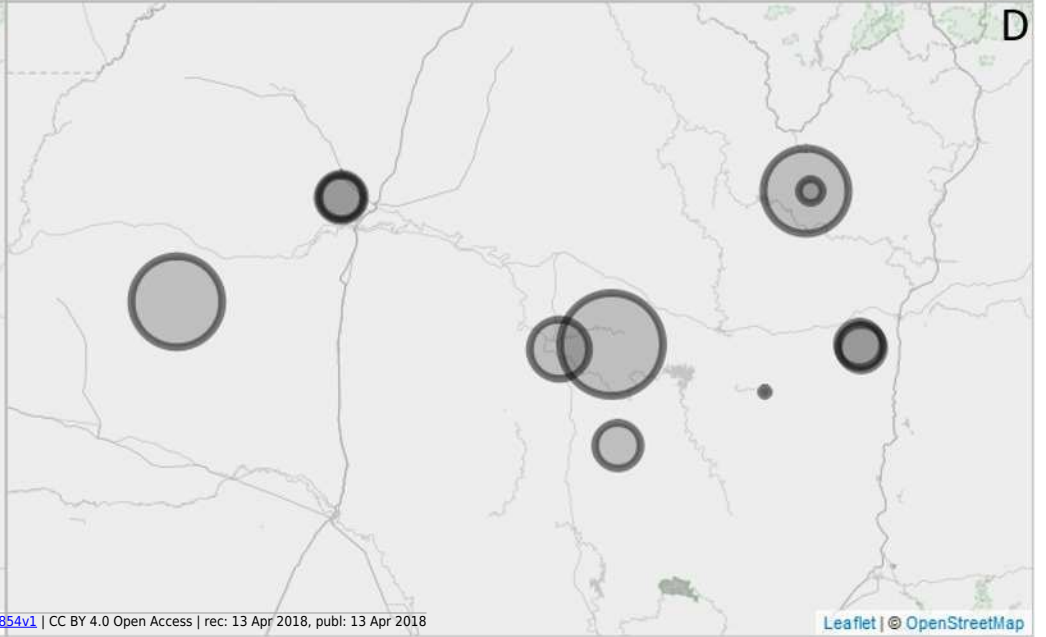
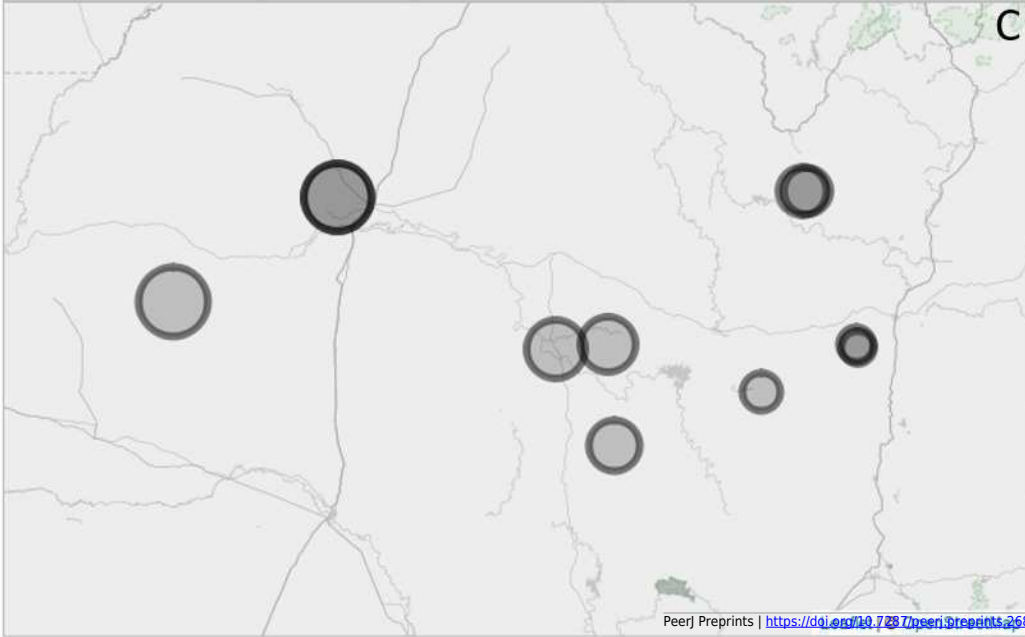
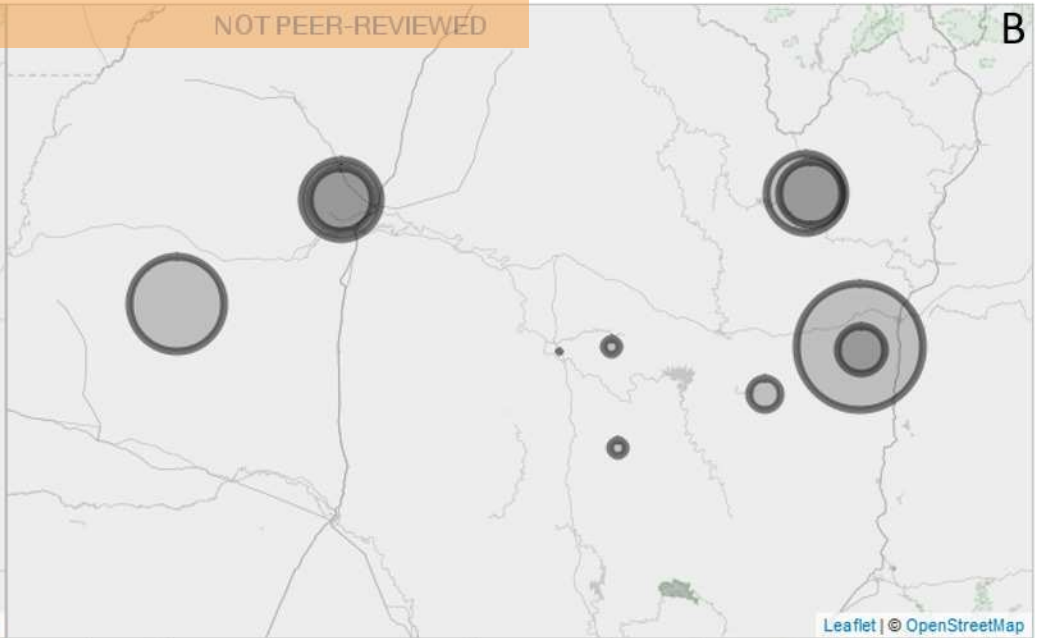
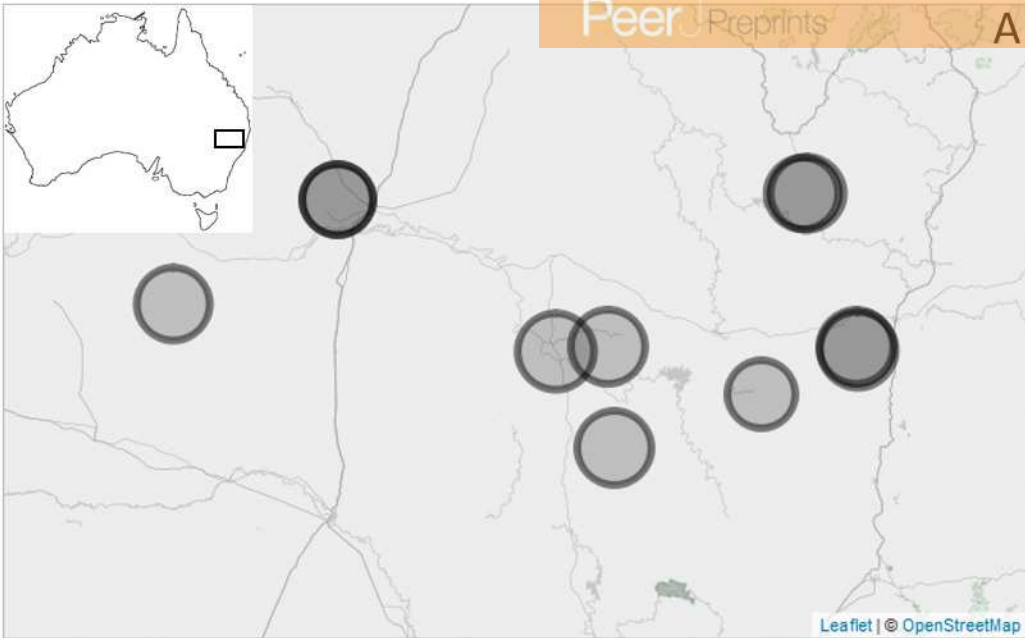
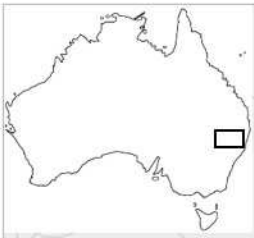


Figure 2 (on next page)

Random effect estimates of model coefficients using Best Linear Unbiased Prediction (BLUP) and 95% confidence intervals of the intercepts and CT_{\max} (centred - Ctmaxc) and CT_{\min} (centred - Ctminc) across sites.

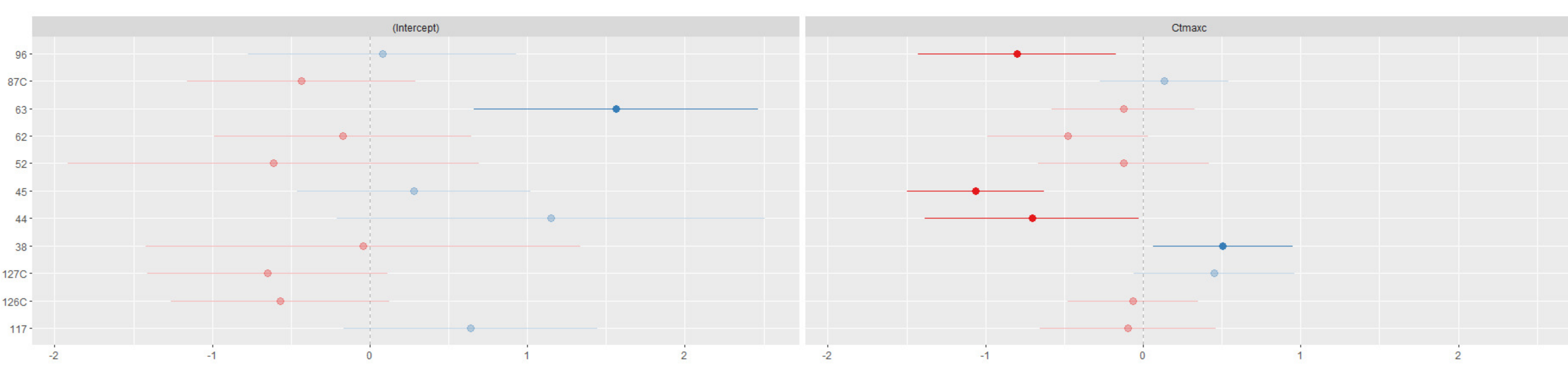
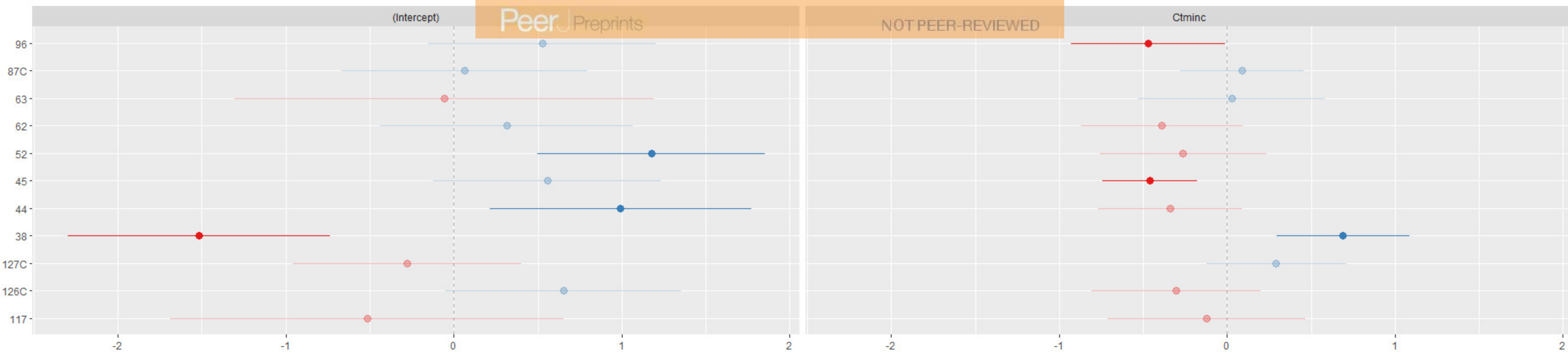


Figure 3(on next page)

95% confidence intervals on the selected CT_{\max} and CT_{\min} models for the standardised factors of Land Use Intensity (LUI) and Clay.

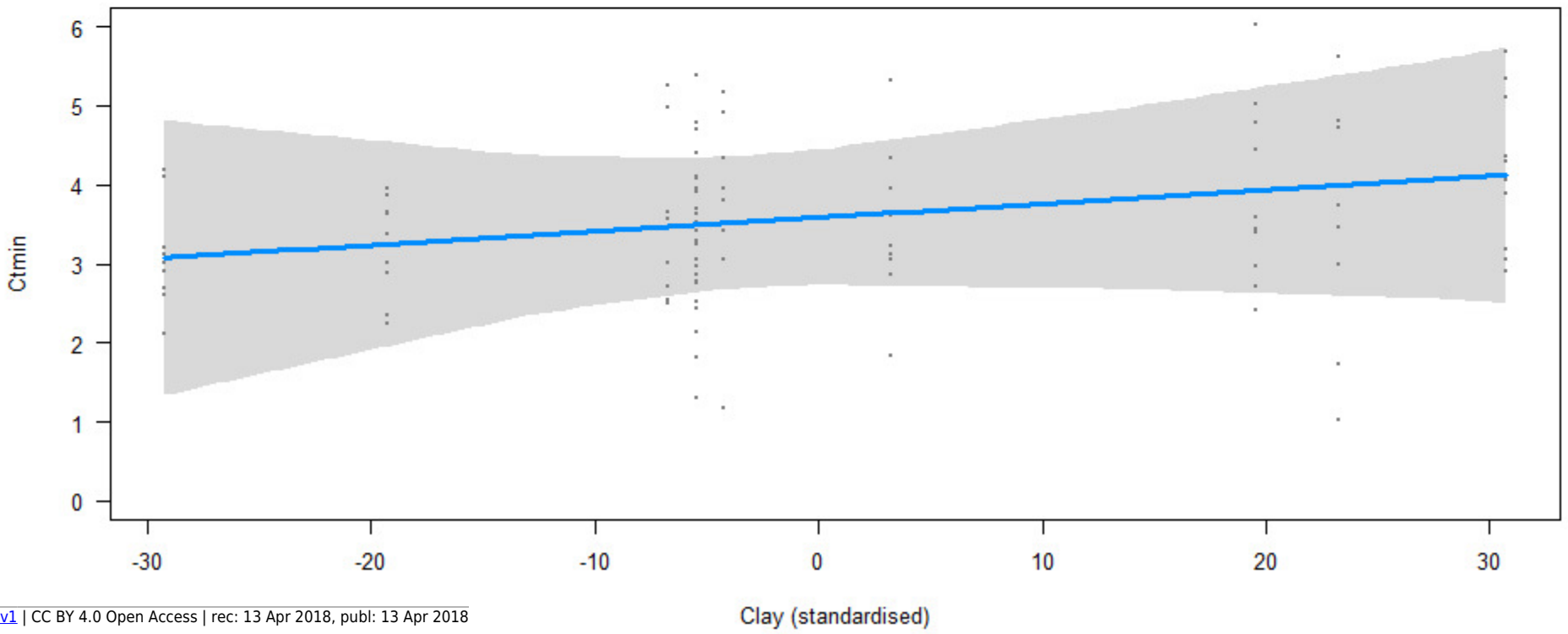
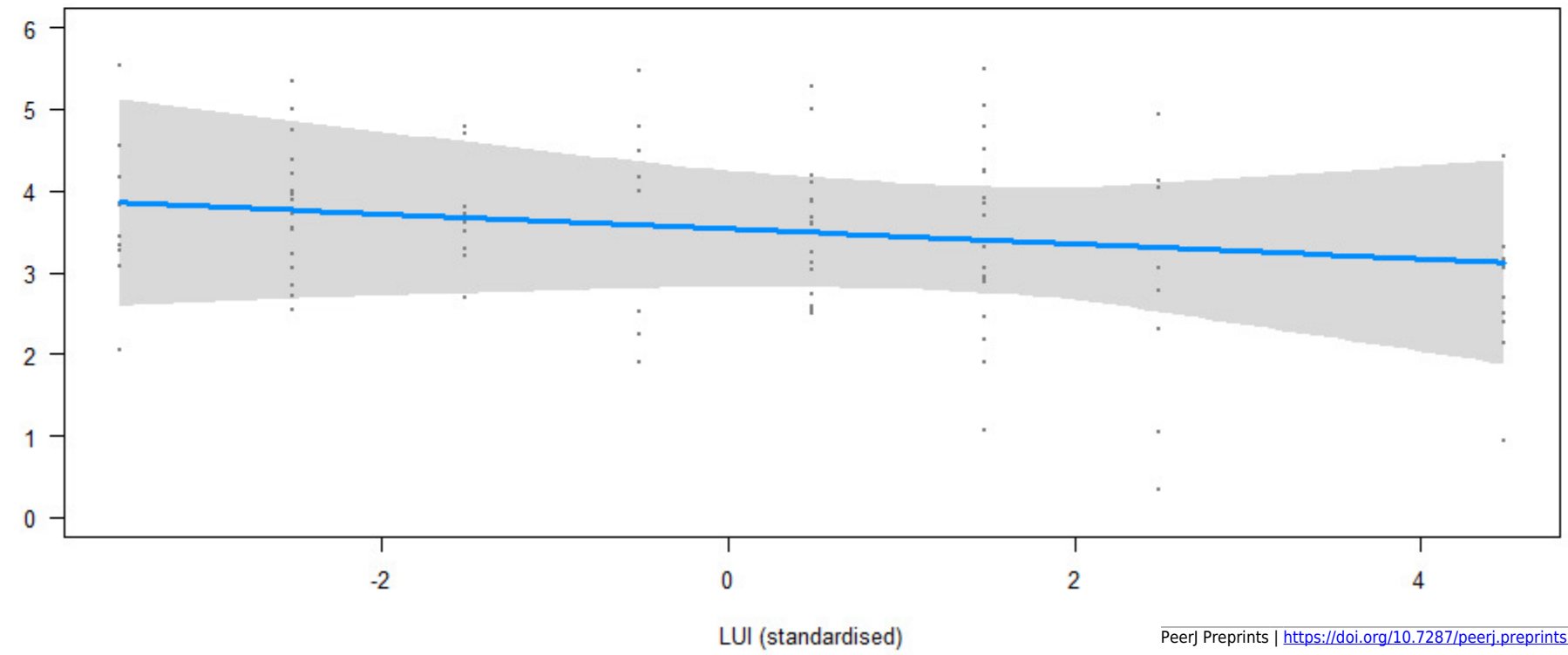
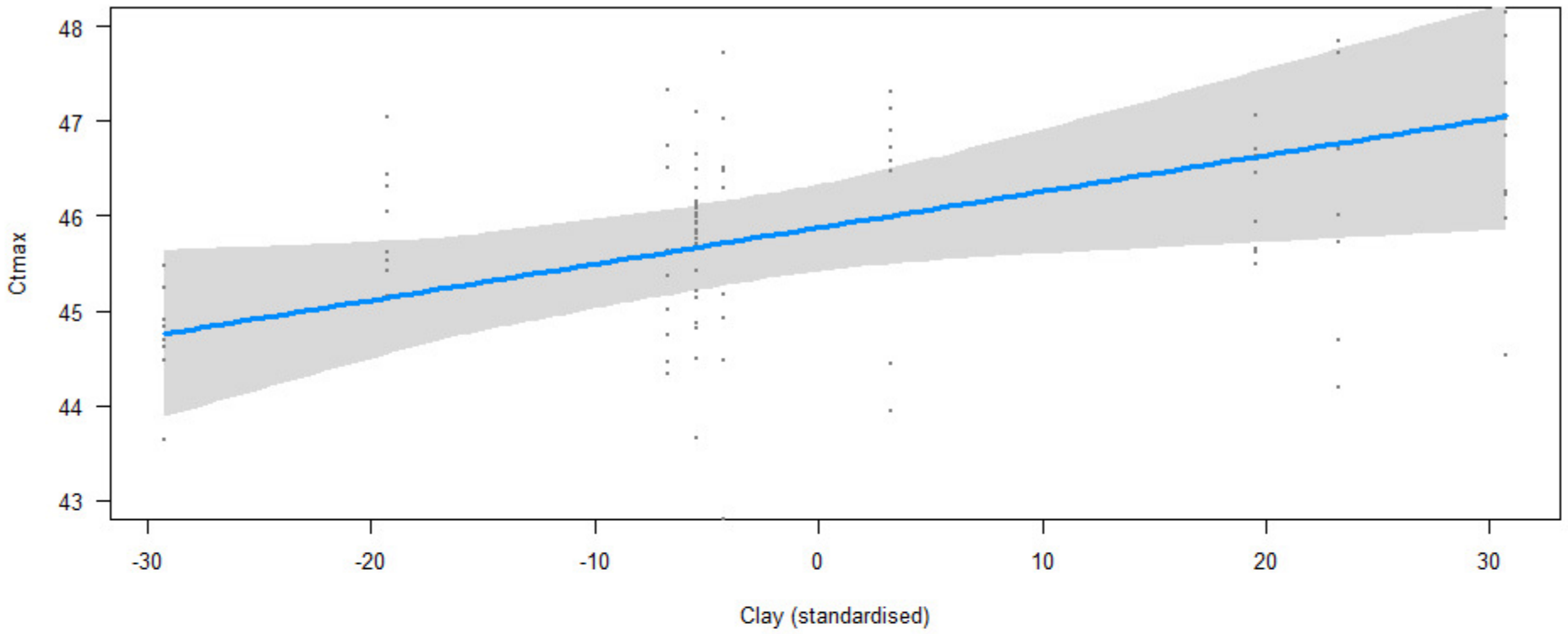
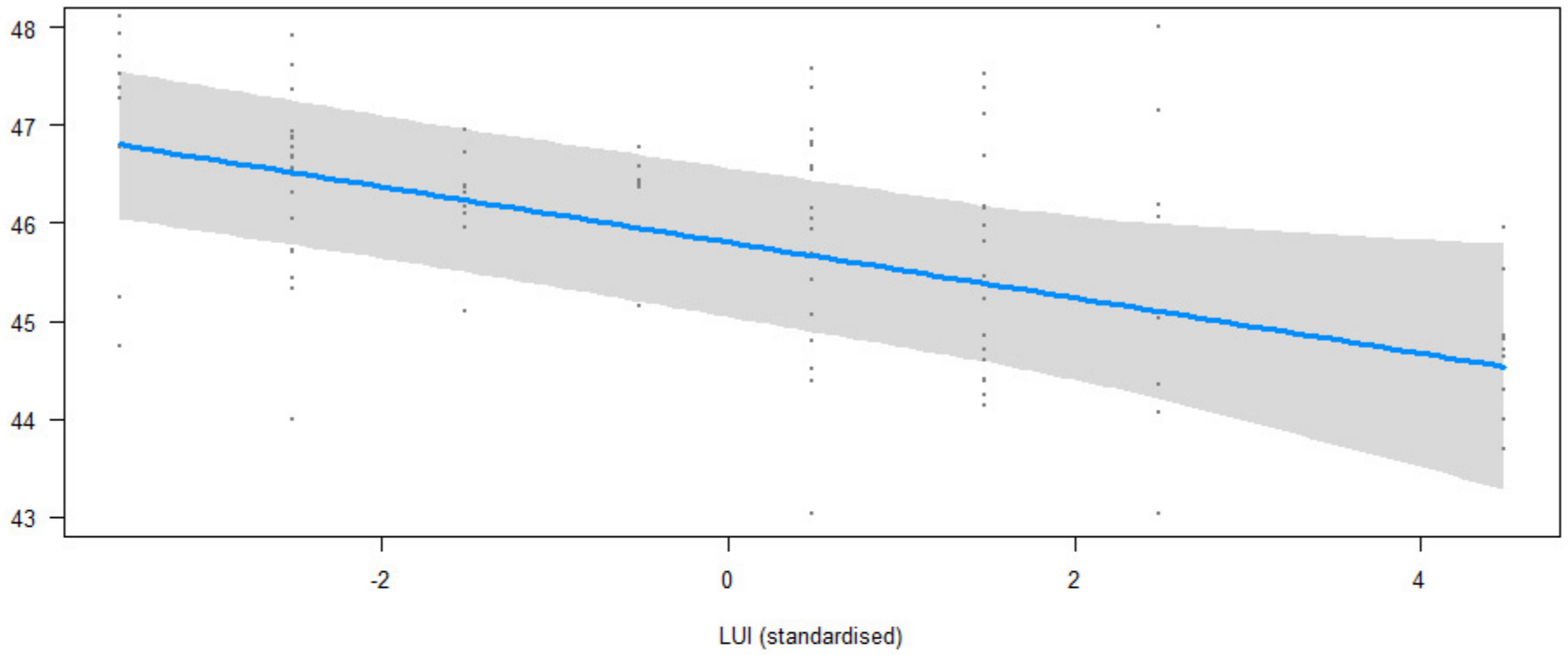


Figure 4(on next page)

Three measures of warming tolerance (3 months, 12 months and 36 months); based on location modelled ANUCLIM data for three different sampling periods) relative to aridity index for each site.

