Urban warming favours C₄ plants in temperate European cities

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Running headline: Urban warming restructures plant assemblages
Summary

1) Elucidating the mechanisms responsible for the structure of urban communities is a key aim of urban ecology, but one that is often confounded by the multitude of environmental changes that are caused by urbanisation. We applied trait-based techniques to identify the specific environmental drivers that shape urban plant assemblages and predict how these drivers will further impact biotas with increasing urbanisation and global environmental change.

2) Urbanised areas across temperate Europe have significantly higher abundance and richness of plants using the C_4 photosynthetic pathway, relative to the total number and species richness of all plant records, than non-urban areas.

3) Urban warming, mediated by the contrasting physiological responses of C_3 and C_4 plants to warming, is the main driver of observed patterns of plant assemblage structure. This mirrors broad-scale and historical distribution patterns of C_3 and C_4 plants. The increased relative abundance of C_4 plants in cities demonstrated here may be indicative of more geographically widespread assemblage changes to be expected in temperate environments under continuing global climate change.

4) Synthesis: Applying a combined trait-based, ecoinformatic and remote-sensing approach provides new insight into the landscape-level consequences of urbanisation. Specifically, we show that localised urban warming in cities across temperate Europe favours C_4 plant species, which respond positively to increased temperatures. Urban plant assemblages are shaped by environmental warming and exhibit significant increases in C_4 plant relative abundance compared to non-urban assemblages.
Keywords: ecophysiology, environmental change, macroecology, photosynthetic pathway, plant–climate interactions, urban heat islands, urbanisation
Introduction

Urbanisation forms a key and growing modern environmental challenge. With an increasing global population, and a rural-urban demographic shift, an ever-increasing number of people live in urbanised areas (United Nations 2015). Urban land cover in 2030 is predicted to be three times the urban area observed in 2000 (Seto, Güneralp & Hutyra 2012), and the ecological impacts of this urban expansion are expected to be substantial and extensive (Grimm et al. 2008; Gaston, Davies & Edmondson 2010; Aronson et al. 2014). At the same time, urbanisation, and the environmental changes that it effects (Seto & Shepherd 2009), present a valuable opportunity to explore fundamental questions in ecology. One such question is how organismal traits mediate environmental variation to structure plant and animal communities (Diamond et al. 2015; Hahs & Evans 2015; Williams, Hahs & Vesk 2015), a significant area of current interest (e.g. Violle et al. 2014; Chown & Gaston 2016).

The urban heat island (UHI) effect is arguably one of the most pronounced and ecologically significant climatic changes caused by urbanisation (Seto & Shepherd 2009; Gaston, Davies & Edmondson 2010). Modifications of the physical environment (see Oke 1981; Seto & Shepherd 2009; Phelan et al. 2015) alter the thermodynamics of an urban system, which generally leads to increases in ambient temperature, relative to surrounding non-urban areas. The magnitude of, and area affected by, this warming is the product of background climatic conditions, population and area of a city, urban planning and cultural factors (Zhao et al. 2014).

Local climatic changes brought about by UHIs are expected to impact urban communities through thermal niche filtering (Williams et al. 2009; Chown & Duffy
Identifying the specific effects of altered climatic variables is, however, confounded by the multitude of other environmental changes associated with urbanisation (Seto & Shepherd 2009). Trait-based methods provide a means to disentangle these confounding effects and identify the specific environmental drivers that shape urban biotas (Williams, Hahs & Vesk 2015). Plants are an ideal group in which to apply trait-based methods to understand the assemblage-level impacts of UHIs due to the well-documented differences in the responses of the C₃ and C₄ photosynthetic pathways to temperature (e.g. Hattersley 1983; Sage & Kubien 2007; Yamori, Hikosaka & Way 2013).

C₃ plants are more tolerant of, and better able to acclimate to, a broad range of temperatures than their C₄ counterparts (Yamori, Hikosaka & Way 2013). Photosynthetic activity increases rapidly in C₄ plants between 20 and 40 °C (Pearcy & Ehleringer 1984) and, at higher temperatures (~40 °C, dependent on [CO₂]), the average photosynthetic rate of C₄ plants is more than double that of C₃ plants (Yamori, Hikosaka & Way 2013). In their controlled plant-growth experiments, Bijoor et al. (2008) noted that biomass of C₄ plants increased substantially in heated plots, demonstrating that increased yield of C₄ photosynthesis under warmer conditions is translated into gains in plant growth. These mechanisms provide a compelling explanation for why ambient temperature has been identified as a key determinant of patterns of C₃ and C₄ plant distributions and relative abundances (Hattersley 1983; Ueno & Takeda 1992; Ehleringer, Cerling & Helliker 1997; Hanba, Kobayashi & Enomoto 2009; Pyankov et al. 2010; Pau, Edwards & Still 2012; Angelo & Daehler 2015). For example, C₄ plant species across Australian grasslands increase in abundance with increasing temperature, while C₃ plants exhibit the
opposite response (Hattersley 1983). Similar geographic patterns have been identified in European (Pyankov et al. 2010) and Japanese (Ueno & Takeda 1992; Hanba, Kobayashi & Enomoto 2009) plant assemblages, and elevation gradients of C₃ and C₄ grasses in Hawai’i have also been attributed to environmental temperature (Angelo & Daehler 2015).

The relatively low photosynthetic yields of C₄ photosynthesis under cool, wet conditions (Sage & Kubien 2007), coupled with the poor freeze-tolerance of C₄ plants (Osborne et al. 2008), mean that C₃ plants dominate plant assemblages across Europe. C₄ plant species account for a small proportion of all plant species (0.51 – 1.50 %; Collins & Jones 1985), or are altogether absent (Ehleringer, Cerling & Helliker 1997) from temperate European assemblages. Urban heat islands may, however, sufficiently alter the local climate such that C₄ plants in urban regions are more productive than those in non-urban areas. Gains in photosynthetic yield (Pearcy & Ehleringer 1984; Sage & Kubien 2007; Yamori, Hikosaka & Way 2013) and biomass (Bijoor et al. 2008) at higher temperatures should enable C₄ plants to have higher fitness, relative to their C₃ counterparts, and could potentially restructure urban plant assemblages. Indeed, it has been suggested that urban warming will favour C₄ plants (Duncan et al. 2011), though no explicit tests of the idea have been conducted. Here, we test the hypothesis that UHI warming restructures plant assemblages and favours C₄ plants in cities across temperate Europe. To do so, we combine trait data on photosynthetic pathways, ecoinformatic approaches (Michener & Jones 2012) using data from the Global Biodiversity Information Facility (GBIF, http://www.gbif.org), and remotely-sensed measurements of UHI warming.
Material and methods

The United Kingdom and Germany were selected as exemplar representatives of the temperate European region, especially given climatic variation between the two countries owing to their more oceanic and continental settings (Peel et al. 2007), respectively. Both countries contain substantial urbanised areas and are extensively sampled with a large number of botanical records in the Global Biodiversity Information Facility (GBIF), which minimises the spatial bias often associated with GBIF data (Yesson et al. 2007; Boakes et al. 2010). Georeferenced occurrence data with ‘no known coordinate issues’ for all plant records in the UK (doi:10.15468/dl.g4lis4; n = 20 477 246) and Germany (doi:10.15468/dl.misihs; n = 5 386 025) were downloaded from GBIF. Although the C_4 photosynthetic pathway only occurs in angiosperms, all plants were included in subsequent analyses to present an overview of changes to whole plant assemblages. Nevertheless, repeat analyses were performed using only angiosperm records to rule out taxonomic bias.

Duplicate records were removed from both datasets and point data were cropped using geographic boundary shapefiles. Counts of all plant occurrence data at a quarter degree resolution (~ 19 km x 28 km) were made. Analyses using this relatively coarse spatial resolution were necessary to compensate for coordinate rounding and minor geopositional errors of GBIF data. Any cell with fewer than 1000 unique points was excluded from further analyses. Urban cells were identified using an urban area shapefile derived from 500 m resolution MODIS data (Schneider, Friedl & Potere 2010), which was rasterised to 0.25 ° resolution. C_4 plants were identified using the list of European C_4 plants compiled by Pyankov et al. (2010) and records from
GrassPortal (Osborne et al. 2014). Any plant identified as using the C4 photosynthetic pathway by either of theses sources was accepted as a C4 species for the purposes of our analyses.

The relative abundance of plants using the C4 photosynthesis pathway was calculated by dividing the number of occurrences of species identified as C4 plants by the total number of occurrence records per 0.25° cell. Species richness of all plants and species richness of C4 plants was calculated by counting the unique instances of species in each cell. The latter was divided by the former to calculate the species richness contribution of C4 plants. All resultant values were expressed as percentages (i.e. multiplied by 100). Relative abundance and species richness contribution of C4 plants in urban and non-urban cells were compared using Mann-Whitney U tests.

The use of C4 plant relative abundance from GBIF data as an approximation of ecological abundance relies on the assumption that sampling and reporting of records is not biased toward either C3 or C4 plants. Although direct bias in favour of either photosynthetic pathway is unlikely, taxonomic bias may indirectly skew abundance estimates. For example, as approximately 80% of known C4 species are monocots (Sage 2016), overrepresentation of this taxon in urban GBIF records may inflate the relative abundance of C4 species such that it is no longer a valid representation of ecological abundances. To ensure that the results of the all-plants analyses were robust to such taxonomic biases, analyses were repeated using monocot and eudicot plant occurrence records separately.
To test if observed patterns of $C_4$ relative abundance were the result of biases arising from sampling effort, a randomisation procedure was used to generate randomised $C_4$ proportion maps based on sampling effort for each quarter degree cell. Pooled species records for each country were randomly redistributed amongst cells based on the sampling effort for each cell (i.e. number of records per cell). From these randomised data the $C_4$ plant proportion for each cell was calculated by dividing the number of newly distributed records of $C_4$ plants by the total record count of each cell. This randomisation procedure was replicated 999 times to generate randomised maps of plant distribution based on sampling effort. Kolgorov-Smirnov tests with Bonferroni-Holme correction were used to compare the 999 maps generated using the randomisation procedure to the maps of observed data.

High-resolution (0.05 °) mean summer (June 2003 - August 2014) daytime and mean winter (December 2003 – February 2015) night-time land surface temperature data for the study areas were extracted from MODIS data layers (MOD11C3; Land Processes Distributed Active Archive Center 2015) and aggregated to 0.25 ° resolution. UHIs were clearly identifiable in these data (Figs. 1a and 2a). The mean summer and winter temperatures of urban and non-urban cells were compared using separate Mann-Whitney U tests for the UK and Germany. To confidently attribute observed patterns of plant assemblage structure to fine-scale (i.e. UHI) climate variation as opposed to background (i.e. regional) climate variation, a null model representing regional climate without localised UHI warming was used. Mean summer and winter climatology data for a 10-year period (June 1996 – August 2005 and December 1995 – February 2005, respectively) were extracted from NCAR’s CESM1.0-CAM5 climate model (cesm.ucar.edu/models/cesm1.0/cam/) and linearly interpolated to 0.25
resolution. A zero-censored tobit regression was fitted to combined C$_4$ relative abundance data for the UK and Germany using only climatology (C$_4$ relative abundance $\sim$ climatology) and using both climatology and remote-sensed MODIS data (C$_4$ relative abundance $\sim$ climatology + MODIS). The relative weight of evidence for each of the models was assessed using the Akaike weight, which provides the probability of each model being the best fit for the observed data (Johnson & Omland 2004). A likelihood-ratio test was used to compare the two fitted regressions and test if regional climate alone can explain the observed patterns.

All analyses were performed in R Statistical Software (R Development Core Team 2015) using the raster (Hijmans & van Etten 2015) and ggplot2 (Wickham 2009) packages.

**Results**

Urban regions of both the UK and Germany were warmer than non-urban regions (Figs. 1a,b and 2a,b; Table 1). Summer temperatures of urban regions of the UK were, on average, 3.47 °C warmer than those of non-urban regions (non-urban median temperature = 17.63 °C, urban = 21.10 °C; Mann-Whitney U test: U = 5289, Z = -9.1659, p < 0.0001, r = 0.3699), though winter temperatures were not significantly different (non-urban median temperature = -1.32 °C, urban = -1.23 °C, U = 16687, Z = -9.1659, p = 0.4969, r = 0.0274). Summer temperatures of urban regions of Germany were, on average, 1.98 °C warmer than those of non-urban regions (non-urban median temperature = 22.06 °C, urban = 24.04 °C, U = 8832, Z = -9.9719, p < 0.0001, r = 0.3624) and winter temperatures were 1.26 °C warmer in urban regions.
than in non-urban regions (non-urban median = -5.28 °C, urban = -4.03 °C, $U = 12288$, $Z = -8.1136$, $p < 0.0001$, $r = 0.2948$).

Plants using the $C_4$ photosynthetic pathway occurred infrequently in records for both the UK and Germany (median relative abundance of each quarter degree cell: 0.0581 % and 0.8372 %, respectively). Urban areas in both countries showed significant increases in $C_4$ relative abundance compared with non-urban areas (Figs. 1b and 2b). For the UK, the non-urban median $C_4$ plant proportion was 0.0424 % and the urban proportion was 0.1818 %; a Mann-Whitney U test confirmed the $C_4$ plant proportions of urban and non-urban cells were significantly different ($U = 7200.5$, $Z = -7.8801$, $p < 0.0001$, $r = 0.3180$). For Germany the non-urban median $C_4$ plant proportion was 0.7867 % and the urban proportion was 1.2619 %; a Mann-Whitney U test confirmed the $C_4$ plant proportions of urban and non-urban cells were significantly different ($U = 11583$, $Z = -8.4930$, $p < 0.0001$, $r = 0.3087$). Plant assemblages of large urban areas had particularly high proportions of $C_4$ plants relative to surrounding non-urban areas (e.g. Greater London, UK, $n_{\text{cells}} = 7$, mean = 0.5742 %, SE = 0.0703 %, adjacent non-urban cells $n_{\text{cells}} = 9$, mean = 0.0161 %, SE = 0.0286 %; Frankfurt/Mainz, Germany, $n_{\text{cells}} = 14$, mean = 1.4225 %, SE = 0.1034 %, adjacent non-urban cells $n_{\text{cells}} = 20$, mean = 0.7615 %, SE = 0.0831 %).

Species richness contribution of $C_4$ plants mirrored relative abundance trends with $C_4$ plants contributing a significantly higher proportion of urban richness than non-urban richness. On average, UK $C_4$ plants accounted for 0.1653 % of species richness in non-urban areas and 0.6515 % of species richness in urban areas ($U = 6278.5$, $Z = -8.5787$, $p < 0.0001$, $r = 0.3462$). In Germany $C_4$ plants accounted for a median of
1.0874 % of non-urban species richness and 1.6654 % of urban species richness (U = 8313, Z = -10.2513, p < 0.0001, r = 0.3726). All patterns of C₄ plant relative abundance and species richness contribution were robust to taxonomic biases and were replicated when angiosperms, monocots, and eudicots were each analysed separately (Table 1, Figs. S1-S4).

Across urban and non-urban cells of both Germany and the UK, *Amaranthus retroflexus* and *Echinochloa crus-galli* were the two most frequently abundant C₄ plant species. Neither of these species are native to Europe, the former is a eudicot and the latter is a monocot. The most frequently abundant C₄ species comprised an approximately equal mix of native and non-native species and the majority were monocots (Table S2). In Germany, the five most frequently abundant C₄ species were shared between urban and non-urban cells. Four out of five of these species were grasses, with *A. retroflexus* being the sole exception. Of the five most frequently abundant species in the UK, three were shared between urban and non-urban cells (*A. retroflexus*, *E. crus-galli*, and *Cyperus longus*).

Although cities were generally better sampled than non-urban areas, randomisation procedures could not replicate observed patterns of relative abundance. All randomised maps were significantly different from the observed distributions of C₄ plant relative abundance (UK, Kolgorov-Smirnov tests: D mean = 0.4083, p-adjusted mean < 0.0001; Germany, Kolgorov-Smirnov tests: D mean = 0.2545, p-adjusted mean < 0.0001; results of pairwise comparisons between observed data and each random permutation in Tables S3 and S4 respectively).
Including high-resolution remotely-sensed temperature data, in which UHIs are identifiable, as an independent variable in a zero-censored tobit regression of C₄ plant relative abundance, significantly improved the model fit over a null model using only interpolated climatology data, which represent regional climate but not localised UHI warming, as an independent variable (log-likelihood of null model = 4759.21, log-likelihood of model using high-resolution climate data = 4927.75; likelihood-ratio test: \( \chi^2(2) = 337.10, p < 0.0001 \); coefficients of each fitted model can be found in Table S5). The magnitude of this improvement was large, with nearly no support for the null model (Akaike weight < 0.0001) and unambiguous support for the model using higher-resolution climate data (Akaike weight > 0.9999; Johnson & Omland 2004). Further confidence can therefore be placed in the UHI effect.

Discussion

In keeping with expectations for temperate climates (Collatz, Berry & Clark 1998; Ehleringer, Cerling & Helliker 1997), C₄ plants had low species richness and relative abundance in plant assemblages across the UK and Germany. Urban areas in both countries did, however, have significantly higher C₄ plant species richness (Figs. S1 and S2) and abundance (Figs. 1c and 2c), relative to total plant richness and abundance respectively, than non-urban areas. Nevertheless, species richness contributions of C₄ plants in urban assemblages remained lower than those reported from naturally C₄-dominated assemblages, such as Australian grasses where C₄ plants contribute toward approximately 60% of total plant richness on average (Hattersley 1983). Coastal regions of the UK also show increased proportions of C₄ plants, in agreement with findings from other temperate regions such as Japan, which were
attributed to the saline soils and increased irradiance generally found in coastal regions (Hanba, Kobayashi & Enomoto 2009). Furthermore, coastal areas are less likely to experience winter temperatures below freezing (Fig. 1b).

Patterns of C₃ and C₄ plant distributions and relative abundances, which randomisation procedures confirmed were not an artefact of sampling bias, are primarily driven by temperature variability at a regional scale (Hattersley 1983; Ueno & Takeda 1992; Ehleringer, Cerling & Helliker 1997; Hanba, Kobayashi & Enomoto 2009; Pyankov et al. 2010; Pau, Edwards & Still 2012; Angelo & Daehler 2015). The background climate variation on which urban areas are overlaid (Zhao et al. 2014; Chown & Duffy 2015) cannot, however, explain the observed patterns of high C₄ relative abundance in urban plant assemblages. Including high-resolution remotely-sensed temperature data, in which UHIs are identifiable, significantly improves the model fit of a zero-censored tobit regression over a null model that represents regional climate but not localised UHI warming. These patterns must, therefore, be the outcome of fine-scale climatic variation, specifically UHI warming, mediated by the strong physiological link between temperature and comparative photosynthetic yields of C₃ and C₄ plants (Pearcy & Ehleringer 1984; Sage & Kubien 2007; Yamori, Hikosaka & Way 2013).

Temperate Europe is perhaps the perfect region in which to identify UHI effects on C₃/C₄ relative abundances. Under mean 2014 CO₂ concentrations of 398.6 ppm (Mauna Loa, Scripps CO₂ Dataset; ~40 Pa under standard atmospheric pressure), 19.5 °C has been identified as the crossover temperature at which C₄ grasses gain a competitive advantage over C₃ counterparts (Collatz, Berry & Clark 1998). In
temperate Europe, average summer temperatures converge around this threshold temperature, with urban areas significantly warmer than non-urban background temperatures due to UHI warming (Figs. 1a and 2a). C₄ plants are, therefore, more likely to have higher relative fitness in urban areas, where warmer environmental temperatures increase photosynthetic yields (Pearcy & Ehleringer 1984; Sage & Kubien 2007; Yamori, Hikosaka & Way 2013), which may also contribute to gains in C₄ plant biomass (Bijoor et al. 2008). Furthermore, freeze-intolerant C₄ plants (Osborne et al. 2008) that inhabit urban areas may be insulated from the harshest winter conditions as UHIs homogenise daily and seasonal temperature variations, and decrease the frequency of extreme cold events (Oke 1981; Gaston, Davies & Edmondson 2010; Phelan et al. 2015). In turn, C₄ plants are represented by more individuals in urban plant assemblages.

Examination of species identity indicated that the most frequently abundant C₄ plant species were largely shared across the extent of both countries and repeated across urban and non-urban areas. Patterns of C₄ plant relative abundance therefore appear to be primarily driven by significant increases, relative to the total number of plants, in the urban abundance of a few common C₄ species rather than plants that are unique to urban regions. Nevertheless, the species richness contribution of C₄ plants was also significantly higher in urban regions, which suggests both common and rare species contribute to the observed patterns. Although the majority of C₄ species identified in both urban and non-urban regions were monocots, when monocots are excluded from analyses patterns of C₄ relative abundance remained constant.
The mechanistic link between $C_4$ photosynthetic yields and temperature means that UHI warming is the most reasonable explanation for the higher than expected proportion of $C_4$ plants found in urban areas. Nevertheless, urbanisation results in multiple climatic changes (Seto & Shepherd 2009) that may also influence plant assemblage structure and contribute to trait-based patterns (Williams et al. 2015).

After temperature, water-availability is thought to be the next-best climatic predictor of $C_4$ plant distributions (Hattersley 1983; Ehleringer, Cerling & Helliker 1997). The extensive use of low-permeability materials and topographical alterations in urban environments give rise to multiple hydrological changes (Gaston, Davies & Edmondson 2010; e.g. alterations to precipitation, surface-water retention, or humidity). These changes are, however, highly variable amongst cities and show positive, negative and neutral responses relative to the background climate (Seto & Shepherd 2009). Furthermore, despite the association between the evolution of the $C_4$ photosynthesis pathway in plants and arid conditions (Sage, Christin & Edwards 2011; Sage 2016), $C_4$ plants exhibit variable responses to water stress and are no more drought tolerant than $C_3$ plants under standard conditions (Ghannoum 2009). It is, therefore, unlikely that water-availability, which varies inconsistently amongst cities, is effecting the trait-based patterns described here.

Physical alterations to the environment brought about by urbanisation processes also have the potential to alter plant assemblage structure (Aronson et al. 2014) but, in contrast to UHI warming, these changes are unlikely to affect $C_3$ and $C_4$ plants in a consistent manner that explains observed patterns. For example, clearing of trees for construction may have decreased the relative abundance of $C_3$ plant species in urban cells and altered lightscapes. $C_4$ photosynthesis is almost completely absent from
trees, generally restricted to the herbaceous layer, and C₄ species perform poorly under fluctuating light (Sage 2016). It has, therefore, been suggested that, in addition to the direct removal of C₃ trees, the absence of dense forests and associated understory environments in urbanised areas may benefit C₄ plants (Kubásek, Urban & Šantrůček 2013). Deforestation is not, however, an exclusively urban practice (e.g. agriculture) and described patterns were also apparent when eudicots were excluded from our analyses (Table 1, Figs. S1b and S2b). Furthermore, lightscapes of urban areas are highly variable and studies examining the responses of plants to urbanisation show positive, neutral and negative interactions for both photophilic and photophobic species (Williams, Hahs & Ves 2015).

Plant assemblage structure of countries with extended histories of human settlement, such as the UK and Germany, are inevitably shaped by horticultural practices, with species exhibiting certain desirable traits favoured over other species. Which plant traits are desirable, however, is highly subjective and varies substantially based on cultural norms and gardener preferences (Kendal, Williams & Williams 2012). Furthermore, gardens are exclusive to neither urban nor non-urban habitats. Should biases toward certain groups of plants exist between urban and non-urban gardens they likely take the form of taxonomic bias (e.g. favouring grasses) rather than direct selection for or against a plant using a specific photosynthetic pathway. Although it is not possible to completely exclude human preference as a contributing factor to the patterns of C₄ relative abundance described here, separate analysis of eudicot and monocot assemblages (Figs. S1-S4; Table 1) has proven that patterns are robust to higher-level taxonomic bias and cannot, therefore, be due to human preference alone. Habitat affinity has also been identified as a factor that may structure plant
assemblages, with plants found in arable fields and settlements characterised by life-history traits that are reflective of the levels of disturbance in their respective environments (Lososová et al. 2006). Whether or not this habitat affinity also exists with respect to physiological traits remains untested. Ultimately, the consistency of the spatial signal of the UHI effect across multiple taxa and differing environments in both an island (UK) and continental setting (Germany) suggests that a more fundamental process than habitat affinity of different suites of plants or gardener preference is responsible for the observed patterns.

Other direct anthropogenic factors must also be considered. Cities are exposed to greater anthropogenic propagule pressure (i.e. propagules introduced directly by human movements) than their non-urban surroundings (Brockerhoff et al. 2014). If, therefore, this pressure is biased toward C₄ plant propagules then it may contribute to the observed increases in C₄ plant relative abundance regardless of urban climate effects. A scenario where propagule pressure from C₄ plants is greater than that from C₃ plants is, however, improbable in temperate Europe. Although many C₄ plants are identified as invasive to the region, and invasive species often exhibit common traits (Thompson & McCarthy 2008; van Kleunen, Weber & Fischer 2010; van Kleunen, Dawson & Maurel 2014; though see Leffler et al. 2014), the C₄ photosynthetic pathway is not common amongst species that are listed as invasive to either the UK or Germany and the most frequently abundant C₄ plants are a mix of native and non-native species (Table S2). Based on invasive species lists complied by the DAISIE European Invasive Alien Species Gateway (europe-aliens.org), only 25 of the 671 genera that incorporate species invasive in the UK, and 17 of the 413 genera that incorporate species invasive in Germany, contain any species identified as C₄ by
either Pyankov et al. (2010) or GrassPortal (Osborne et al. 2014). Propagule pressure exerted on cities is, therefore, more likely to be biased toward C₃ rather than C₄ plant propagules. The hypothesis that observed patterns are the result of the urban environment being better suited than non-urban environments for the establishment and growth of C₄ plants, be they native or invasive, is more plausible.

Future research

Previously, decoupling UHIs, which are one of the most general environmental changes brought about by urbanisation, from other urbanisation impacts has proven difficult. Approaches that combine ecoinformatic and trait-based methods, such as those that have been applied here, are a plausible solution to this problem (Diamond et al. 2015; Hahs & Evans 2015; Williams, Hahs & Vesk 2015). They also provide a powerful addition to ecological toolsets, and hold great promise for understanding how environmental variation structures assemblages and communities in response to all forms of anthropogenic change. Indeed, they offer a novel means to give effect to the proposal that urban ecosystems lend themselves to tests of predicted ecological responses to climate change (Youngsteadt et al. 2014; Diamond et al. 2015).

In this specific instance, the identification of UHI warming as a significant driving factor of urban patterns of C₄ plant relative abundance provides unique insight into the way in which other plant assemblages might be re-structured with on-going global climate change. Recent observations of increases in the relative abundance of C₄ plants in North American grasslands have been attributed to contemporary climatic warming (von Fischer, Tieszen & Schimel 2008) and it is anticipated that broad-scale patterns of C₃ and C₄ plant distribution and abundance will be substantially changed
under future climate conditions (Angelo & Daehler 2015). Based on the plant
assemblage patterns identified in this study across urban regions, relative abundance
of C₄ plants will increase in plant assemblages across temperate Europe as the region
warms over the next century. Other ecological responses to UHI warming may also be
indicative of the more widespread changes that are to be expected as global
temperatures rise. The warming caused by UHIs has already been described altering
the phenology of both plants (Zhang et al. 2004; Neil & Wu 2006; Gazal et al. 2008)
and insects (Townroe & Callaghan 2014; Diamond et al. 2014) and comparable
phenological shifts should be anticipated at global scales under continuing climate
change.

In conclusion, the application of trait-based ecoinformatic methods has provided new
insight into the effects of urbanisation on temperate European plant assemblages.
Although they remain rare, C₄ plant species represent a significantly larger proportion
of plant abundance in urbanised areas than they do in non-urban areas. We have
attributed this pattern to warming driven by the urban heat island effect. This
hypothesis is supported by physiological differences between C₃ and C₄ plants, which
exhibit contrasting responses to environmental warming.

Acknowledgements
We thank Petr Pyšek, Melodie McGeoch and three anonymous reviewers for their
comments on a previous version of the manuscript and all members of the ChownLab
for discussion of this work. This work was supported by Australian Research Council
Discovery Project DP140101240.
Data Accessibility

Occurrence record data are freely available from the GBIF database (www.gbif.org; UK, doi:10.15468/dl.g4lis4; Germany, doi:10.15468/dl.misihs). Data used for all display items and results of randomisation tests are presented in supplementary information.
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Table 1: Results of pairwise Mann-Whitney U tests comparing urban and non-urban environmental temperature and plant assemblage structure of the UK (urban \(n_{\text{cells}} = 64\), non-urban \(n_{\text{cells}} = 550\)) and Germany (urban \(n_{\text{cells}} = 81\), non-urban \(n_{\text{cells}} = 676\)).

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</tr>
<tr>
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<td>0.0424</td>
<td>7200.5</td>
<td>-7.8801</td>
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<td>0.3180</td>
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<tr>
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<td>0.0780</td>
<td>8199.5</td>
<td>-7.1231</td>
<td>&lt;0.0001</td>
<td>0.2875</td>
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<tr>
<td>Monocots</td>
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<td>0.0748</td>
<td>7206.0</td>
<td>-8.0698</td>
<td>&lt;0.0001</td>
<td>0.3257</td>
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<td>Eudicots</td>
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<td>0.0310</td>
<td>10228.5</td>
<td>-5.6885</td>
<td>&lt;0.0001</td>
<td>0.2296</td>
</tr>
<tr>
<td><strong>C_{4} richness contribution (%)</strong></td>
<td></td>
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<tr>
<td>All plants</td>
<td>0.6515</td>
<td>0.1653</td>
<td>6278.5</td>
<td>-8.5787</td>
<td>&lt;0.0001</td>
<td>0.3462</td>
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<td>Angiosperms</td>
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<td>0.2551</td>
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<td>0.3340</td>
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<td>0.4796</td>
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<td>Eudicots</td>
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<td>0.1336</td>
<td>9060.5</td>
<td>-6.5898</td>
<td>&lt;0.0001</td>
<td>0.2659</td>
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<td><strong>Germany</strong></td>
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<tr>
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<td>22.0620</td>
<td>8832.0</td>
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<td>-5.2833</td>
<td>12288.5</td>
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<td><strong>C_{4} relative abundance (%)</strong></td>
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<td>0.2923</td>
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<tr>
<td><strong>C_{4} richness contribution (%)</strong></td>
<td></td>
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<td>1.0874</td>
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Fig. 1. Average summer (a) and winter (b) temperature (2000 – 2015) and relative abundance of C₄ plants as a proportion of all plant occurrence records in GBIF (c) for the United Kingdom at 0.25 ° spatial resolution. Cells classified as urban (shaded) were significantly warmer in summer than non-urban cells and had a significantly higher proportion of C₄ plants (Table 1).

Fig. 2. Average summer (a) and winter (b) temperature (2000 – 2015) and relative abundance of C₄ plants as a proportion of all plant occurrence records in GBIF (c) for Germany at 0.25 ° spatial resolution. Cells classified as urban (shaded) were significantly warmer than non-urban cells and had a significantly higher proportion of C₄ plants (Table 1).
Table S1: Results of all analyses for each quarter degree cell. Row names are: xy = coordinates of cell centre, urban_bin = binary classification of urban/non-urban cell, plant_count = count of all plant occurrences for cell, plant_sr = all plants species richness, c4_count = count of all C4 plants, c4_sr = species richness of all C4 plants, eudi_count = count of eudicots, eudi_sr = species richness of eudicots, mono_count = count of monocots, mono_sr = species richness of monocots, eudi_c4_count = count of C4 eudicots, eudi_c4_sr = C4 eudicot species richness, mono_c4_count = count of C4 monocots, mono_c4_sr = C4 monocot species richness, angio_count = count of angiosperms, angio_sr = angiosperm species richness, angio_c4_count = count of C4 angiosperms, angio_c4_sr = C4 angiosperm species richness, day_summer_temp = MODIS mean daytime summer temperature (2000 – 2015), night_winter_temp = MODIS mean nighttime winter temperature (2000 – 2015), cesm_summer = mean summer temperature from CESM GCM, cesm_winter = mean winter temperature from CESM GCM.

Table S2: Taxonomic data and Native status in Europe for the five most frequently abundant C4 plant species in urban and non-urban cells of the UK and Germany. † recently reclassified as Kali spp., though this is placement is contested (http://dx.doi.org/10.12705/635.15).

Table S3: Results of Kolgorov-Smirnov tests for the UK. Pairwise comparisons between observed data and each random permutation.

Table S4: Results of Kolgorov-Smirnov tests for Germany. Pairwise comparisons between observed data and each random permutation.
Table S5: Model fits of zero-censored tobit regressions of C₄ plant relative abundance (% against a null model using only interpolated climatology data, which represent regional climate but not localised UHI warming, and an Urban heat island (UHI) model incorporating high-resolution remotely-sensed temperature data, in which UHIs are identifiable. Log-likelihood of null model = 4759.21, log-likelihood of UHI model = 4927.75. A likelihood-ratio test confirmed that the UHI model represented a significant improvement in model fit (χ²(2) = 337.10, p < 0.0001).
Fig. S1. Relative abundance of C₄ plants as a proportion of angiosperm (a), monocot (b) and eudicot (c) plants based on occurrence records in GBIF for the UK at 0.25 ° spatial resolution. Proportions of urban cells (shaded) were significantly different from those of non-urban cells (Table 1).

Fig. S2. Relative abundance of C₄ plants as a proportion of angiosperm (a), monocot (b) and eudicot (c) plants based on occurrence records in GBIF for Germany at 0.25 ° spatial resolution. Proportions of urban cells (shaded) were significantly different from those of non-urban cells (Table 1).

Fig. S3. Species richness contribution of C₄ plants as a proportion of richness of all plants (a), monocots (b) and eudicots (c) based on occurrence records in GBIF for the UK at 0.25 ° spatial resolution. C₄ richness contributions for urban cells (shaded) were significantly different from those of non-urban cells (Table 1).

Fig. S4. Species richness contribution of C₄ plants as a proportion of richness of all plants (a), monocots (b) and eudicots (c) based on occurrence records in GBIF for Germany at 0.25 ° spatial resolution. C₄ richness contributions for urban cells (shaded) were significantly different from those of non-urban cells (Table 1).

Fig. S5. C₄ plant relative abundance against mean daytime summer (a) and night-time winter (b) temperatures for the UK. Line represents LOESS smoothed curve with 95 % confidence intervals in grey.
Fig. S6. C₄ plant species richness against mean daytime summer (a) and night-time winter (b) temperatures for the UK. Line represents LOESS smoothed curve with 95% confidence intervals in grey.

Fig. S7. C₄ plant relative abundance against mean daytime summer (a) and night-time winter (b) temperatures for Germany. Line represents LOESS smoothed curve with 95% confidence intervals in grey.

Fig. S8. C₄ plant species richness against mean daytime summer (a) and night-time winter (b) temperatures for Germany. Line represents LOESS smoothed curve with 95% confidence intervals in grey.