The role of deterministic succession during forest development within a southern African savanna

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ABSTRACT

Woody encroachment can lead to a switch from open savannas to dense woodlands or forests. This has implications for both the composition of ecological communities and the provision of ecosystem services such as nutrient cycling and grazing capacity. The patterns and underlying drivers responsible for woody encroachment are not fully understood. Here, we investigate the underlying determinants of bush clump formation (a form of encroachment) in a South African savanna and explore whether bush clump succession is driven by deterministic (i.e. predictable changes in species composition) or stochastic (i.e. random) processes. Specifically, we test (1) whether the similarity in species composition of saplings and trees differs among small and large clumps, (2) which environmental factors are driving succession, and (3) if forest specialization of tree and sapling species within bush clumps increases with the successional gradient. Similarity in species composition between saplings in small clumps and trees in large clumps was higher than similarity between trees in small clumps and trees in large clumps. Furthermore, temperature, soil moisture, relative humidity and light intensity were related to changes in species composition along the successional gradient. As expected, forest specialization of trees increased with increasing clump area indicating that late-successional bush clumps have more forest-type species. The directional changes of species found along the successional gradient suggest a deterministic process of succession driven by changes in local environmental conditions during clump formation.

1. INTRODUCTION:

Woody encroachment, i.e. the increase in density of indigenous woody cover within grassy ecosystems, is a major concern globally (Archer et al., 2017). Associated changes in tree-grass ratios modify the composition of ecological communities (Ratajczak, Nippert & Collins, 2012) and the provision of ecosystem services (Asner et al., 2004) and is thought to be the result of a combination of both local (e.g. changes in fire and herbivory management) and global (e.g. increased atmospheric carbon dioxide concentrations) drivers (Wigley, Bond & Hoffman, 2010, Skowno et al., 2017).

Although encroachment is generally viewed as the densification of woody species, it can also occur through the formation of bush clumps (i.e. dense clumps of woody species) which subsequently expand in size (O'Connor & Chamane, 2012). This succession often starts with the establishment of a founder tree which then facilitates the establishment of other species (Bews, 1917, Barnes & Archer, 1996, Dean, Milton & Jeltsch, 1999). Bush clumps have been recorded from various localities in southern Africa, West Africa, and Texas (USA) (e.g. Bews, 1917, Mordelet, Abbadie & Menaut, 1993, Archer, 1995, Northrup et al., 1995, Hester, Scogings & Trollope, 2006, O'Connor & Chamane, 2012), where they are usually initiated by single woody species (except Bews, 1917).

Two underlying processes, namely deterministic and stochastic, are recognized in succession (Dent, DeWalt & Denslow, 2013, Dini-Andreote et al., 2015). Deterministic succession involves a directional change in species composition which is driven by environmental conditions that allow establishment and persistence (Peterson & Carson, 2008, Dent, DeWalt & Denslow, 2013). In deterministic succession, factors such as site conditions,

species sorting mechanisms and disturbance intensity may directionally influence the trajectory of succession (Meiners et al., 2015, Chang & Turner, 2019). Thus species benefit from each other, forming characteristic associations of species rather than random species associations (Liautaud et al., 2019). For instance, along a forest successional gradient, species composition can change predictably due to increases in soil moisture as the canopy of a forest becomes denser (Schönbeck et al., 2015). Accordingly, the sapling composition of early successional stages may be similar to that of the adult composition in later successional stages as these saplings ultimately grow to become adult trees in later successional stages (Dent, DeWalt & Denslow, 2013). Furthermore, tree composition during early successional changes can be expected to be different from that of the sapling community in later successional stages, indicating poor establishment of early successional species in later successional stages (Dent, DeWalt & Denslow, 2013). In contrast, stochastic succession as an inherent probabilistic biological process, is influenced by less predictable factors such as random chance events, the availability of colonisers in the surrounding landscape, or unique conditions during disturbance events (Zhou et al., 2014). For example, processes that govern a species' ability to disperse into an area, and thus species availability, are largely random (Meiners et al., 2015). Such processes can lead to changes in species composition that are not strongly associated with the environmental conditions at a particular site (Dini-Andreote et al., 2015).

One result of deterministic succession in bush clumps would be that older bush clumps are dominated by late-successional forest species (Jarvel & O'Connor, 1999, O'Connor & Chamane, 2012). As light levels decline during succession (Montgomery & Chazdon, 2001), shade-intolerant species should disappear and shade-tolerant (i.e. forest) species increasingly dominate in old (i.e. large) bush clumps (O'Connor & Chamane, 2012). Moreover, old bush

clumps should differ in other microclimatic conditions from young bush clumps, e.g. maximum

air temperature and irradiance may be lower while humidity may be higher in old vs. young bush

clumps (Jarvel & O'Connor, 1999). Such changes in microclimate during bush clump succession

can reduce the establishment of herbaceous species (Archer, 1995), reduce the flammability of

vegetation and provide a suitable environment for fire-intolerant (forest) species to establish.

Hence, bush clump succession and associated changes in microclimate are likely to favor forest

species which can be expected to establish in later stages of succession.

Another prediction of deterministic bush clump succession is that more specialized

species will colonize, survive, and dominate in old, large clumps. For instance, plant species may

be specialized in terms of their climatic or habitat niche breadths (Dolédec, Chessel & Gimaret-

Carpentier, 2000), i.e. the range of (micro-)climatic and habitat conditions they can tolerate. The

level of specialization across species in a community can thus provide insights into whether

different successional stages are associated with the presence of particular plant groups (e.g.

forest specialists). Measures of habitat breadth (Barnagaud et al., 2012) would indicate the ability

of a species to survive in a number of different ecosystems (e.g. savannas or forests), and

whether species that come at later stages of succession are unable to tolerate a wide range of

habitat conditions compared to early-successional species. Therefore, it can be expected that

bush clump succession leads to an increase of forest specialists and a decrease of species with

wide habitat niches (Christensen & Peet, 1984, Palmer & Dixon, 1990).

Studies on bush clumps have suggested that succession of woody species within bush

clumps occurs through directional change (Bews, 1917, Archer et al., 1988, Archer, 1990,

O'Connor & Chamane, 2012). However, most studies (except O'Connor & Chamane, 2012) have

neither empirically tested this nor have they systematically compared the association between

multiple environmental factors and different successional trajectories (Meiners et al., 2015). Hence, elucidating whether the pattern of species turnover is deterministic or stochastic can inform on the drivers and processes of bush encroachment across space and time.

Here, we investigate the successional patterns associated with the development of bush clumps within a South African open savanna. Woody encroachment increases tree-grass ratios in savannas and can lead to a transition into forest. Under encroachment, savanna species, both woody and herbaceous, eventually disappear (Ratajczak, Nippert & Collins, 2012), and productivity and function of the landscape changes (O'Connor, Puttick & Hoffman, 2014). We test whether species composition changes with clump size, how multiple environmental factors in bush clumps relate to changes in species composition, and whether habitat specialisation of species is more dominant in large bush clumps. Specifically, we test whether (1) similarity between saplings of small bush clumps and trees of large bush clumps is higher than the similarity between trees of small bush clumps and trees of large bush clumps, indicating deterministic succession (Dent, DeWalt & Denslow, 2013); (2) changes in species composition between bush clumps are accompanied by changes in microclimatic conditions, namely temperature, relative humidity, soil moisture, and light availability (Dent, DeWalt & Denslow, 2013); (3) species composition changes towards more forest-type species as clumps get larger at the expense of savanna-type founder individuals; and (4) species with narrow habitat breadth are more common in old or large bush clumps (Palmer & Dixon, 1990). Our study provides novel insights into how multiple environmental variables accompany species turnover during succession of South African bush clumps which can inform management of woody encroachment and conservation of savannas and grasslands.

2. METHODS

2.1 Study site

Our study was conducted in Buffelskloof Nature Reserve (25º19'22.21'' S, 30º29'15.41'' E), in NE South Africa (Table 1; Figure S1a). The reserve was proclaimed in 1980 around a 10 km long, 300–800 m wide forested valley (Figure S1b). Much of the grasslands and savannas surrounding the reserve have been afforested with pines. The first pine plantations were planted in the 1950's. Before proclamation of the reserve, the areas in and around the valley were burned annually during April–May to protect the surrounding plantations. Since the reserves establishment, a two-year fire rotation has been employed with burning during May and early June (John Burrows pers. comm.). Three major biomes (and vegetation types) are represented in the reserve: forest (Northern Afrotemperate Forest), grassland (Lydenburg Montane Grassland) and savanna (Lydenburg Thornveld) (Mucina & Rutherford, 2006). Savannas are open in structure, with trees in a matrix of grasses. Within the savannas of this reserve, there is evidence of recent woody thickening (Haddad, 2011) which often occurs through the formation of bush clumps, i.e. associations of one or more large woody species (> 1.2 m; hereafter referred to as 'trees') with woody saplings (< 1.2 m; hereafter referred to as 'saplings') growing beneath the canopy of the trees and separated from other such clumps by grassy vegetation (Figure S1 c & d). A number of herbivores can be found in the reserve (Table 1).

TABLE 1. Table summarizing the characteristics of the study area, Buffelskloof Private Nature Reserve, which is situated in north-eastern South Africa.

Characteristic	Description
Area (ha)	1 500
Altitude (m.a.s.l)	1 000 – 1 800
Mean Annual Precipitation (mm)	1 270 (Burrows & Burrows, 2003)
Mean Annual Temperature (ºC)	16.3 (Climate-data.org, 2019)
Climate	Warm, wet summers, and cold, dry winters with periodic frost events.
Geology	The geology of the reserve consists largely of sedimentary and subordinate volcanics of the Transvaal Supergroup (Mucina & Rutherford, 2006)
Common Herbivores	Chacma baboons *(Papio ursinus)* and various antelopes (e.g. greater kudu (*Tragelaphus strepsiceros*), common duiker (*Sylvicapra grimmia*), red duiker (*Cephalophus natalensis*), mountain reedbuck (*Redunca fulvorufula*) and oribi (*Ourebia ourebi*)).

2.2 Vegetation sampling

Sampling was carried out between 2015 and 2017 (July-August 2015, January, April-May, July 2016, January, March, and August 2017). While small saplings were detected, no evidence of seedling germination (e.g. cotyledons) was found. Most small saplings appeared to be in a 'stunted' sapling stage, which can last for several decades until suitable conditions allow for rapid growth into mature trees (Antos, Guest & Parish, 2005). Therefore, season of sampling is unlikely to have influenced species composition.

We randomly chose 40 clumps in the reserve to cover a range of bush clump sizes. Clump size was determined by measuring the length and breadth of each clump (to the nearest 10 cm) using a measuring tape and calculating its area as an ellipse. The smallest clump was defined

as a single tree (> 1.2 m) beneath which woody vegetation (< 1.2 m) was present. Across the reserve, the number of small and intermediate size bush clumps outnumber very large clumps; as such, there are a greater number of smaller sized clumps than there are large clumps. Within each clump, all tree species (i.e. species listed in Coates Palgrave (2002)) were identified to species level.

The circumference of each tree (defined as individuals > 1.2 m) was recorded at 30 cm above the ground using a sewing tape measure (to the nearest mm). The measurement height of 30 cm was selected as the clumps are often too dense to consistently measure diameter at breast height. Additionally, a count of the number of saplings (defined as individuals < 1.2 m) of each species was made per clump. The 1.2 m cut-off to distinguish between saplings and trees was chosen, which is in the range of other studies that have differentiated between saplings and trees in forests (Janse-Ten Klooster, Thomas & Sterck, 2007). This cut-off is lower than that for savanna trees (2-4 m, Higgins, Bond and Trollope (2000)): bush clump saplings would have been exposed to lower, or no, flames; also, a higher cut-off would have resulted in too few trees for comparing sapling and tree assemblages between small and large clumps. We assumed that the largest tree(s) (in circumference) in the bush clumps was (were) the founder tree(s) (i.e. the initial tree around which the bush clump established). The trunks of the founders were always considerably larger than those of other trees in the bush clumps.

Basal area was estimated for each tree stem (for multi-stemmed trees, the area was calculated per stem), using the formula $A = C^2 / 4\pi$, where A is basal area and C is the circumference of the stem measured 30 cm from the tree base. Total woody basal area per clump was calculated by summing the areas of all stems and was used as a proxy for bush clump

maturity. In addition, a measure of tree and sapling species richness (number of species), and a count of trees (trees > 1.2m), were calculated for each clump.

2.3 Environmental data

Temperature, relative humidity, soil moisture and light intensity were measured in each clump. Temperature and relative humidity measurements were recorded using Maxim iButton dataloggers that were placed in the center of clumps from 21 November 2018 to 29 March 2019. Of the 40 iButtons, 17 were hygrochrons (measuring both temperature and relative humidity), whereas the remaining 23 iButtons were thermochrons (measuring temperature only). While humidity was not recorded in all clumps, the 17 hygrochrons were placed in a range of clump sizes to measure the range and variability of humidity across the successional gradient (Table S1). All iButtons were programmed to capture data at 60-minute intervals at a $0.0625°C$ resolution. Four humidity loggers were faulty or got lost. Soil moisture was recorded for each clump using a FieldScout TDR 300 portable soil moisture meter (Spectrum Technologies, Inc., Plainfield, IL, USA) on 19 and 20 January 2016.

For soil moisture, four readings were taken beneath the canopy of each clump (one reading in each quarter of the clump). Average, maximum, and minimum soil moisture was calculated per clump. Light intensity was recorded using a ceptometer (Accu-PAR LP-80, Decagon Devices, Pullman, WA, USA) in May 2019. Four light readings were recorded both inside and outside the clumps. The difference in these readings was then averaged and used as a measure of light intensity. All readings were taken between 9h00 and 15h00 on cloud-free days.

2.4 Statistical analyses

To test the assumption that bush clump size could be used as a proxy for age, three separate generalized linear regression models (GLMs) were used to investigate the relationship between bush clump area and a) species richness (a Poisson distribution with a log link), b) woody basal area (a Gaussian distribution with an identity link), and c) the total number of trees (a Poisson distribution with a log link). We expected that species richness, woody basal area, and the total number of trees would increase with an increase in clump size and thus clump maturity.

To compare similarity of sapling and tree species composition between large and small clumps we identified the 10 smallest clumps and the 10 largest clumps as 'small' and 'large', respectively. The Morisita-Horn index, which is robust to uneven sampling (Magurran, 2013), was used to quantify similarity in species composition between small and large clumps. The Morisita-Horn similarity index was calculated for each combination of large and small clumps. We tested if the similarity in species composition between trees-in-large-clumps and saplings-in-small-clumps was greater than the similarity between trees-in-large-clumps and trees-in-small-clumps. These two sets of similarity indices were compared between small and large clumps using a Welch two-sample t-test (Welch 1947).

To test whether environmental variables accompanied the change in species composition with succession, we tested whether, for the 30 largest bush clumps, differences in similarity with the 10 smallest clumps could be explained by environmental conditions in the largest clumps. We expected that increased dissimilarity of large bush clumps with small bush clumps would be accompanied by directional changes in the microclimate. Initially, similarity in species composition between all combinations of the 10 smallest and the 30 largest bush clumps was calculated using the Morisita-Horn index of similarity. For every large bush clump the similarity

with the 10 smallest clumps was averaged, providing 30 data points (one for each of the 30 largest bush clumps). We then tested whether this average similarity index of the small bush clumps with each of the 30 largest bush clumps could be explained by mean and minimum temperature, range in relative humidity, minimum soil moisture (the lowest value identified out of each of the 4 measures taken per clump) and light intensity of the 30 largest bush clumps (Appendix A, table A1). As the number and identity of the 30 largest bush clumps with measures of temperature ($n = 27$), relative humidity ($n = 13$), soil moisture ($n = 30$), and light intensity ($n = 30$) differed (Table S1), four univariate GLM models were run. Models were fitted with a quasi-binomial distribution with a logit link as the response variable was bound between zero and one and over-dispersed (Zuur et al., 2009).

An index of habitat preference for each species was developed using habitat descriptions from several southern African tree field guides (Coates Palgrave, 2002, Schmidt, Lotter & McCleland, 2002, Van Wyk & Van Wyk, 2013). The habitat index ranged from 1 (open habitat species) to 8 (closed habitat species) (Table S2/3).

To test whether the proportion of a) founder tree species decreased, and b) forest tree species increased with increasing clump size, we fitted beta regression models (a Gaussian distribution with an identity link) using the proportion of founder/forest tree species per bush clump as a response variable and bush clump size as predictor variable.

To test whether forest specialists were more abundant in large bush clumps, a community-weighted mean of forest specialization was calculated for each bush clump using the equation: $\sum_{i=1}^{s} P_i \times FS_i$, where s is species richness, P_i is the proportion of species i and FS_i is the forest specialization value for species i (Table S3). High values represent bush clumps with higher numbers of forest specialist species. GLMs (a Gaussian distribution and an identity link)

were used to test if the community-weighted mean of forest specialization increased with bush clump size (part 2). Analyses were repeated for both trees and saplings.

To test whether there was a difference in average forest specialization across the clump gradient, mean forest specialization for species of the 10 smallest clumps, the 10 largest clumps and of surrounding savanna species was calculated. Savanna species were categorized as those species growing in a grassland matrix with no woody species growing beneath their canopy in the study site, based on *ad hoc* observations. These species were also described as being found in closed and open woodlands in the habitat preference index. Identification as savanna trees was also confirmed by expert opinion (John Burrows pers. comm.). One-way ANOVA with Tukey post-hoc tests were used to assess whether the mean forest specialization differed between small clumps, large clumps, and the savanna habitat. Analyses were run separately for trees and saplings.

Habitat breadth was calculated for each species by summing the number of habitat classes (from the habitat preference index; Table S2/3) for which the species was described. This value was used as an indication of habitat specialization with species ranging from specialists (habitat breadth = 1; occur in only one habitat class) to generalists (habitat breadth = 8; occur in only all 8 habitat classes). This was used to calculate a measure of community-weighted mean of tree species habitat breadth using the equation: $\sum_{i=1}^{s} = Pi \ x \ HBi$, where s is species richness, Pi is the proportion of species and HBi is the habitat breadth for species i (Table S3).

To assess whether species in large bush clumps possessed narrower habitat breadths, GLMs (fitted with a Gaussian distribution and an identity link) were used to test if the community-weighted mean of tree species habitat breadth (response variable) changed with bush clump size (predictor variable). We expected that old, large bush clumps would show a greater

proportion of habitat specialists. Additionally, we compared the habitat specialization of savanna species (not considered in the above analysis) with the habitat specialization of trees in small and large bush clumps. For this, the range of habitat breadth values for species of trees in the ten small bush clumps, the ten large clumps and the surrounding savanna species were calculated. One-way ANOVA, followed by Tukey post-hoc tests, were used to assess whether species habitat breadth of trees was significantly different between small bush clump species, large bush clump species and savanna species. One-way ANOVA and Tukey post-hoc tests were repeated to assess whether species habitat breadth of saplings was significantly different between small bush clump species, large bush clump species and savanna species.

All analyses were conducted in R, version 3.5.1 using packages: *vegan* (Oksanen et al., 2007), *corrplot* (Wei et al., 2017), *car* (Fox et al., 2012), and *hdi* (Dezeure et al., 2015).

3. RESULTS

A total of 105 tree species (totaling 21,471 woody individuals) were identified across the 40 bush clumps, of which 24 (22.9%) were identified as founder individuals (Table S2). All 105 species occurred at least once as trees (> 1.2 m), whereas only 92 species were recorded as saplings (< 1.2 m) across the clumps. Bush clump area ranged from 10 m^2 to 1343 m^2 (mean = 266 m^2, SE = 347.7 m^2). Total basal area per bush clump varied between 0.2 m^2 and 30.2 m^2 (mean = 5.5 m^2, SE = 7.4 m^2). The total number of trees per bush clump ranged between one and 350 (median = 14), and saplings between four and 3,503 (median = 131). The average distance between bush clumps and the forested valley was 188 m (SE = 91.2).

As expected, with an increase in bush clump area, species richness (Figure 1a), basal area (Figure 1b) and the number of trees (Figure 1c) increased (Table S4). Consequently, we assumed that bush clump size was a proxy for bush clump maturity.
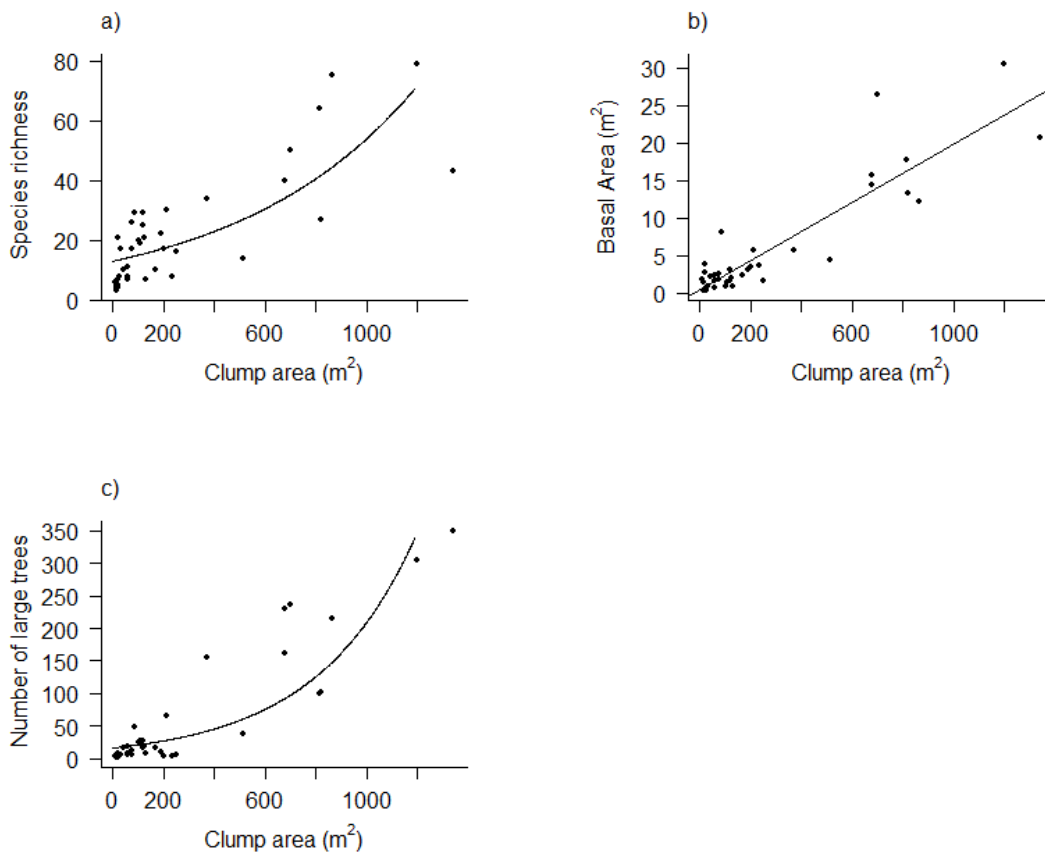

FIGURE 1 Results from generalised linear models testing the relationships between bush clump area and (a) species richness ($R^2 = 0.68$, $F(38,39) = 79.74$, $P = 7.191\text{e-}11$), (b) basal area ($R^2 = 0.82$, $F(38,39) = 170.81$, $P = 1.233\text{e-}15$), and (c) the number of large trees within each bush clump ($R^2 = 0.76$, $F(28,39) = 205.88$, $P = 2.2\text{e-}16$).

As expected, the species composition of trees in large bush clumps more closely resembled the species composition of saplings in small bush clumps than the composition of trees in small bush clumps (Figure 2b).
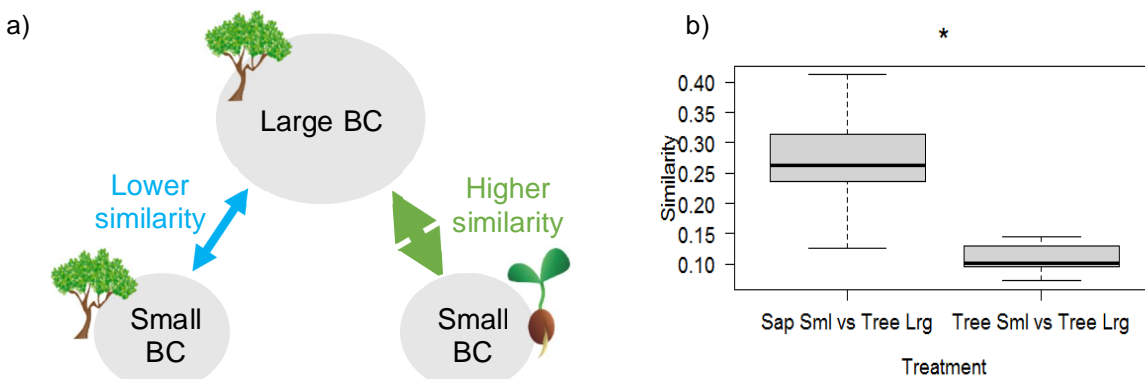

FIGURE 2 For systems undergoing deterministic succession, it could be expected that a) the similarity of adult tree species composition of large bush clumps more closely resembles similarity of sapling composition in small bush clumps than that of adult tree composition in small bush clumps. (b) Boxplots illustrating the similarity in species composition (calculated as the Morisita-Horn similarity index) between different sized bush clumps at Buffelskloof Private Nature Reserve, South Africa. As expected, the similarity of the adult tree composition of large bush clumps more closely resembles that of saplings in small bush clumps than that of adult trees in small bush clumps ($t = 9.29$, $P = 5.004\text{e-}09$). The boxplots illustrate the lower (Q1) and upper (Q3) quartiles. The plots also illustrate the mean and median observation for similarity. Data falling outside of the Q1-Q3 range are plotted as outliers. Abbreviations: Sap = sapling; sml = small; lrg = large, BCs = bush clumps.
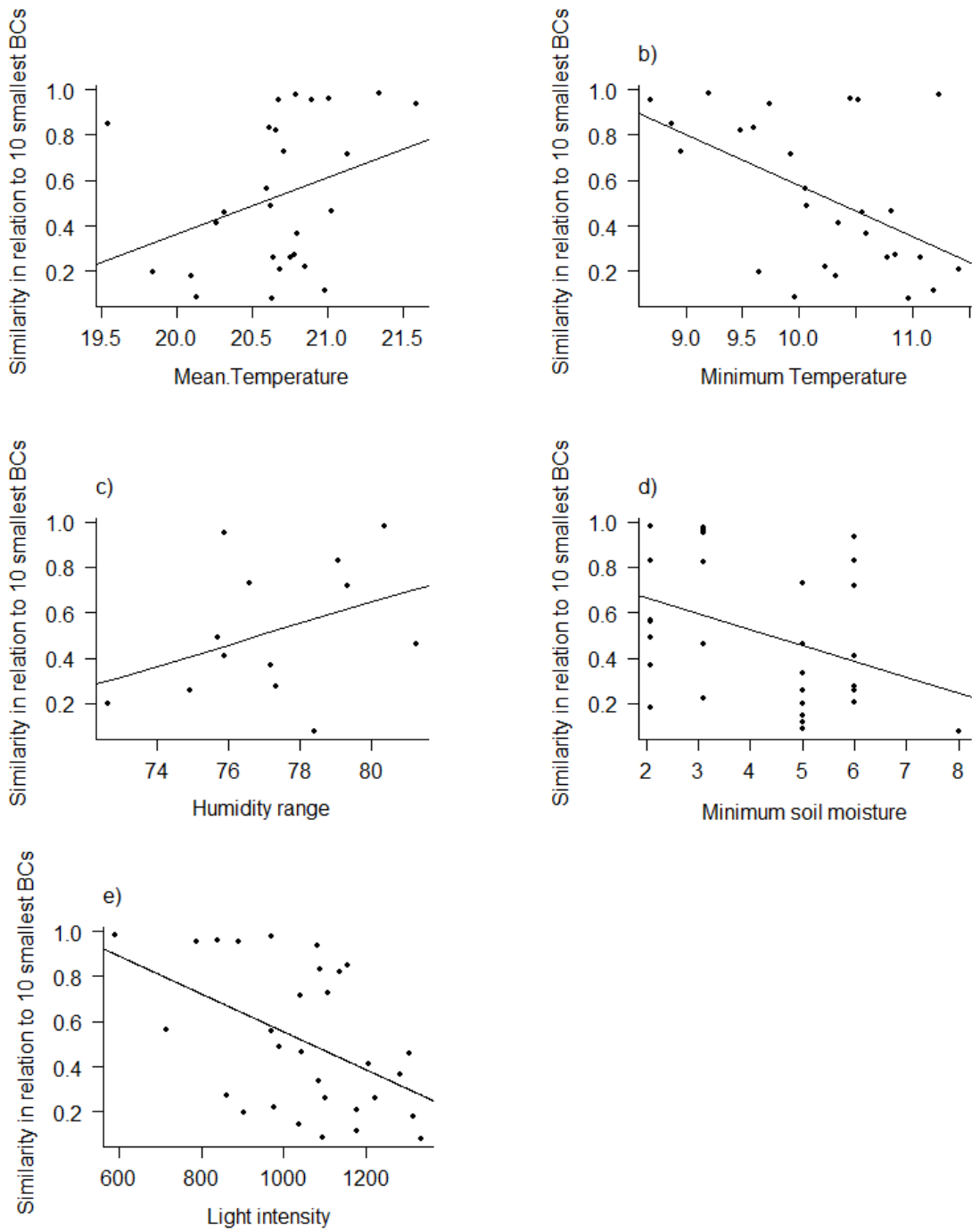
FIGURE 3 Partial residual plots illustrating the relationships between environmental variables and similarity in species composition. Similarity is quantified as the average Morisita-Horn index of similarity in species composition between each of the 30 largest bush clumps with the 10 smallest bush clumps.

Similarity was used as a response variable and environmental variables (mean and minimum temperature, the range of relative humidity, minimum soil moisture and light intensity) as predictors. The panels show how similarity in tree species composition is related to a) mean temperature ($R^2 = 0.10$, $F(25, 26) = 3.14$, $P = 4.60e-02$), (b) minimum temperature ($R^2 = 0.25$, $F(25, 26) = 9.36$, $P = 5.00e-03$), (c) range of relative humidity ($R^2 = 0.13$, $F(11, 12) = 1.892$, $P = 4.30e-02$), (d) minimum soil moisture ($R^2 = 0.12$, $F(28, 29) = 4.525$, $P = 4.10e-02$), and (e) light intensity ($R^2 = 0.20$, $F(28, 29) = 8.17$, $P = 8.00e-03$). The y-axis can be interpreted as small bush clumps illustrating high similarity to the 10 smallest bush clumps (high similarity values on the y-axis) whereas large bush clumps would share low similarity with the 10 smallest bush clumps (low similarity values on the y-axis). BCs = bush clumps.

Changes in species composition similarity between the largest bush clumps and the small bush clumps were predicted by changes in all measured environmental variables (Figure 3; Table S5). As similarity with smallest bush clumps decreased (i.e. species composition of large bush clumps became more dissimilar with small clumps), mean temperature decreased (Figure 3a), minimum temperature increased (Figure 3b), the range of relative humidity decreased (Figure 3c), minimum soil moisture increased (Figure 3d), and the difference in light intensity increased (Figure 3e).

The proportion of forest species significantly increased with an increase in bush clump size (part 1; Figure 4a; Table S6), while the proportion of founder species decreased (Figure 4b; Table S6). Furthermore, the community-weighted mean (part 2) of a) tree forest specialization (Figure 5a; Table S7) and, b) sapling forest specialization (Figure 5b; Table S7) increased with bush clump size. Tree forest specialization did not significantly differ between small bush

clumps, large clumps and savanna trees (Figure 5c). Sapling forest specialization did

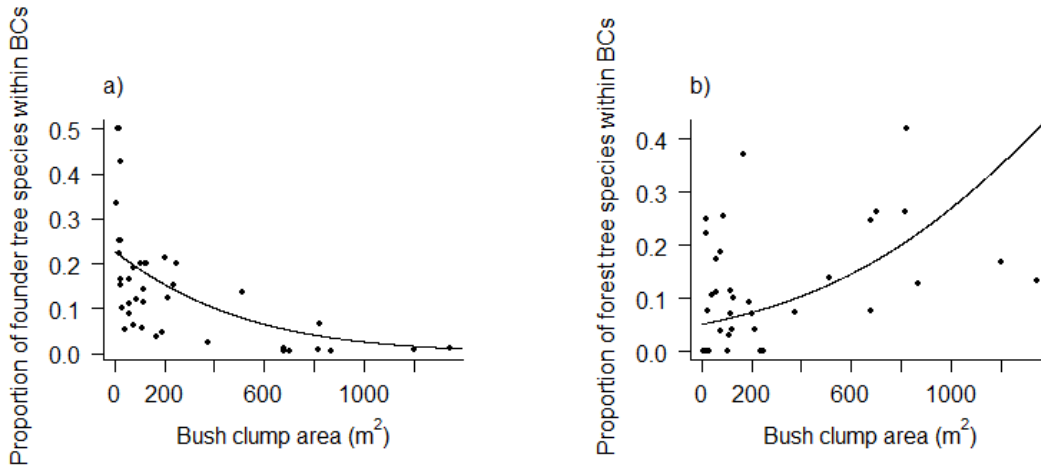significantly differ between small bush clumps, large clumps and savanna trees (Figure 5d).

FIGURE 4 Relationship between bush clump area and (a) the proportion of founder tree species ($R^2 =$

0.61, $z = 4.33$, $P = 1.48e-05$), and (b) the proportion of forest tree species ($R^2 = 0.15$, $z = 4.41$, $P = 1.03e-$

05). Founder trees were identified as those species that most likely initiated a bush clump whereas forest

species were identified using field guides. BCs, bush clumps.

Species with narrow habitat breadths were expected to be common in large clump.

However, the community-weighted mean of habitat breadth of both trees ($R^2 = 0.03$; Table S7)

and saplings ($R^2 = 0.03$; Table S7) showed no relationship with bush clump size. The mean

habitat breadth of trees ($F(2, 198) = 2.69$, $P = 7.00e-02$) and saplings ($F(2, 191) = 1.61$, $P =$

2.04e-01) did not differ between small bush clumps, large bush clumps and the savanna group.
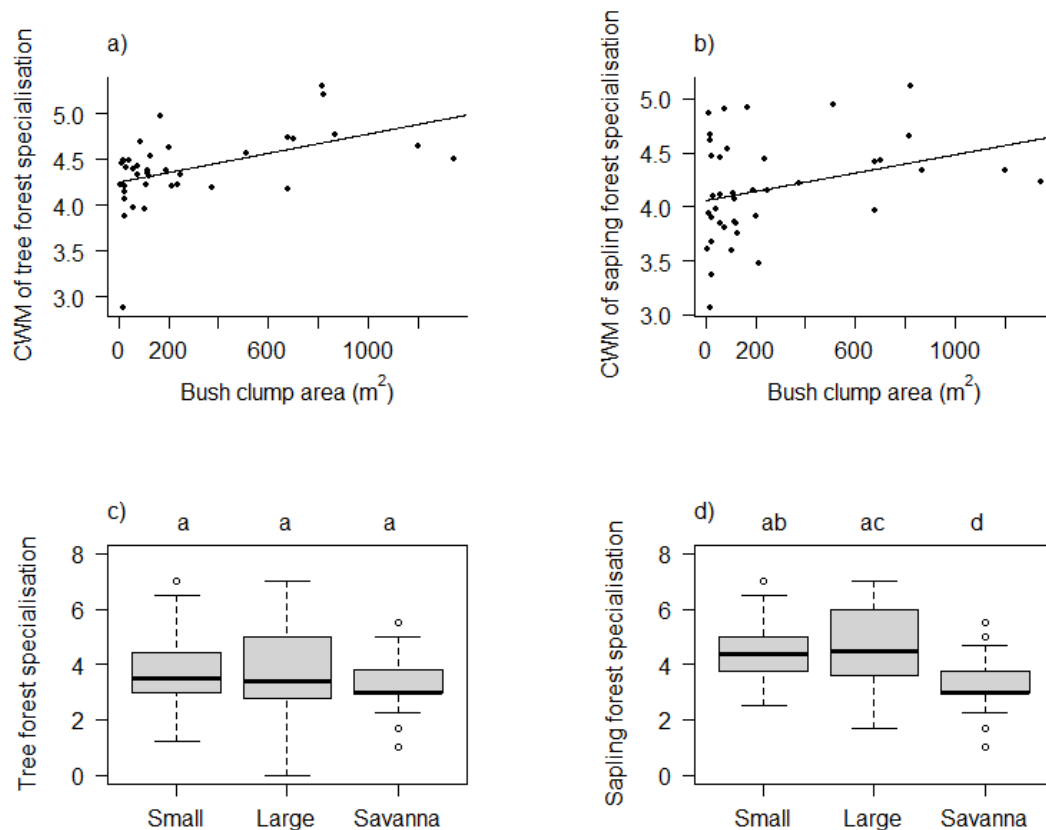
FIGURE 5 Forest specialization of species in different sized bush clumps. (a) Results of a generalized linear model illustrating how the community-weighted mean of forest specialization of trees changes with bush clump size ($R^2 = 0.22$, $F(38,39) = 10.88$, $P = 2.12\text{e-}03$). (b) Changes in the community-weighted mean of forest specialization of saplings with bush clump size as derived from a generalized linear model ($R^2 = 0.10$; $F(38,39) = 4.24$, $P = 4.63\text{e-}02$). Boxplots showing forest specialization of saplings in small bush clumps, large bush clumps and in the surrounding savanna, respectively. (c) Tree forest specialization did not significantly differ between small bush clumps, large clumps and savanna trees ($F(2, 199) = 2.34$, $P = 9.90\text{e-}02$); (d). Sapling forest specialization did significantly differ between small bush clumps, large clumps and savanna trees ($F(2, 1993 = 29.92$, $P = 4.82\text{e-}12$). The boxplots illustrate the lower (Q1) and upper (Q3) quartiles. The plots also illustrate the mean observation for forest specialization. Different letters represent significant differences. Data falling outside of the Q1-Q3 range

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are plotted as outliers. Abbreviations are as follows: Small = small bush clumps; large = large bush

clumps; Savanna = surrounding savanna.

4. DISCUSSION

Our results suggest that woody encroachment is initiated by the establishment of a founder

individual which then facilitates the establishment of other species, resulting in a directional

change towards forest-type species in larger and older bush clumps. This change in species

composition is apparently driven in part by alterations in microclimatic environmental conditions

as the bush clump develops and vegetation thickens.

The similarity in species composition between saplings of small bush clumps and trees of

large bush clumps was higher than the similarity between trees of small bush clumps and trees of

large bush clumps, suggesting that saplings arising from the seed bank of small bush clumps

grow to become the large trees in large bush clumps. This is an indication of deterministic

succession. Whether a successional trajectory follows a directional path can be affected by

factors such as dispersal constraints, site conditions, and disturbance intensity (Cramer, 2007,

Norden et al., 2009, Meiners et al., 2015, Chang & Turner, 2019). In particular, a barrier to

dispersal or variation in dispersal ability (Dent, DeWalt & Denslow, 2013) may lead to spatial

aggregation, increasing dissimilarity in species composition between adjacent sites. However,

this does not seem to apply within this study system. Here, the Afrotemperate forest and savanna

biomes exist in close proximity (average distance between each of the 40 bush clumps and the

forest edge was 188 ± 91 m with a range of 363 m) and most bush clump species are fleshy-

fruited (Jamison et al., 2017), suggesting an endozoochoric dispersal mechanism (Sinnott-

Armstrong et al., 2018). Therefore, dispersal from the forest (seed source) to founder trees,

which provide perches and shade for avian and mammalian (e.g. baboon) dispersers, is unlikely to be prevented. Seeds that germinate beneath the canopy of founder trees then lead to further expansion of the clumps (Bews, 1917, O'Connor & Chamane, 2012).

Our study highlights the importance of multiple environmental factors for the deterministic development of bush clumps within a savanna system as changes in species composition were accompanied by changes in microclimate (temperature, relative humidity, soil moisture) and light availability. Larger clumps had lower mean temperatures and higher minimum temperatures. Warmer temperatures increase seedling growth rates, which can allow seedlings to escape the fire trap more rapidly (Wakeling, Cramer & Bond, 2012). Bush clumps also had a regulating effect on humidity, i.e. variability in humidity decreased with clump size, and this was associated with a change in species composition. Higher stability in humidity reduces evapotranspiration rates beneath the canopy while simultaneously increasing the moisture content (Jarvel & O'Connor, 1999). Moreover, increased soil moisture in larger clumps, possibly related to decreased evaporation under denser canopies in large clumps (Zhu et al., 2013), favors the establishment of forest trees (Pastor & Post, 1986). Lastly, lower light intensity, as found in larger bush clumps, is an important compositional driver during succession as late-successional species, which usually possess tough, long-lived leaves that efficiently utilize light and are slow-growing and shade-tolerant, are best suited to such conditions (Bazzaz & Pickett, 1980, McCulloh et al., 2011). Thus the change in community composition of the bush clumps is characterized by those species that can reach the bush clump, establish and persist under the particular environmental and ecological conditions of the clumps (Peterson & Carson, 2008).

Changes in these environmental drivers with increasing bush clump size suggest that directional changes in species composition in bush clumps are brought about by autogenic processes (Archer et al., 1988, O'Connor & Chamane, 2012); changes in the microclimate of the bush clumps form a positive feedback system allowing the persistence and expansion of the clumps. Early successional species establish poorly in late successional stages where resources have been altered (Pacala & Rees, 1998) by the bush clump canopy. Instead, early successional species are replaced with late successional species which are adapted to new levels of resources such as reduced light intensity (Pacala & Rees, 1998). Therefore, species composition moved towards more forest-type species as clumps got larger.

While many bush clump studies subjectively infer deterministic succession (Bews, 1917, Archer et al., 1988, Archer, 1990), few empirically test this process (though see O'Connor & Chamane, 2012). Our study shows empirical evidence for directional change in species composition with succession, even in a system where the potential tree species pool is large (the 1500 ha reserve supports 216 woody species, see Buffelskloof Nature Reserve (2020)). Additionally, our study system differed from most others (except Bews (1917)) in that the clumps were initiated by several species rather than a single species (Table S2). While founder trees grew in a matrix of grasses, several founder species were not typical savanna species but rather forest edge species (Table S2). Nevertheless, founder trees were not associated with termite mounds, rocky areas, or any other features that apparently promotes the establishment of these trees. Thus, the specific factors allowing trees to establish in a grassy matrix remain to be understood. Additionally, what drives tree seedlings to establish under these species and not under most 'true' savanna species in the landscape (e.g. *Pterocarpus angolensis*, *Combretum molle*, and *Heteropyxis natalensis*) remains unknown.

The average habitat breadth of species in bush clumps showed no trend with succession. We suggest that this is because savanna species are also specialists. Savanna systems, like other grassy ecosystems, have distinct suites of species that are adapted to the unique conditions and disturbances that are integral to savanna ecosystems (Bond & Parr, 2010). This also supports the result that several species can be ranked according to the stage of succession in which they are most likely to occur (Table S7).

Forest–savanna boundaries are generally determined by natural disturbances (e.g. fire and herbivory) characteristic of savanna biomes but absent from forest biomes (Hoffmann, Orthen & Franco, 2004). In the absence, or reduced frequency or intensity of these disturbances, the savanna biome can switch to forest (Sankaran, Ratnam & Hanan, 2008). Adaptations of savanna trees, e.g. deciduousness, thick bark, resprouting mechanisms, allow them to persist within disturbance-driven savannas (Joubert, Smit & Hoffman, 2012). In comparison, forest trees are generally fire-sensitive and less resistant to water stress (Hoffmann, 2000, Hoffmann, Orthen & Franco, 2004). Consequently, forest saplings are scarcely found within savannas (Hoffmann et al., 2012). However, within the sheltered microclimate of a bush clump, forest trees are protected from fire and can outcompete savanna saplings as they have denser foliage (Hoffmann et al., 2012) and are more shade tolerant (Montgomery & Chazdon, 2001). We propose that the low flammability of trees compared to grasses keep fires out of bush clumps and lower fire intensity at the edge of bush clumps (O'Connor & Chamane, 2012), thus enabling bush clump persistence, and, in the case of infrequent or cool fires, expansion and concomitant species have turnover within the sub-canopy of the bush clumps. Once forest species have established, development towards canopy closure is faster than if only savanna species were present (Hoffmann et al.,

2012) because forest trees having higher leaf area, which translates into denser crowns (Gotsch et al., 2010) and reduced light levels (Schönbeck et al., 2015).

As approximately 25% of the Earth's land surface is comprised of savanna biomes, a better understanding of how woody encroachment proceeds is of global importance (Wigley, Bond & Hoffman, 2010, Stevens et al., 2017). Woody encroachment alters the species composition and therefore significantly contributes to biodiversity degradation in savanna ecosystems. Deterministic succession is often seen as a process leading to a "climax" system (Guariguata & Ostertag, 2001, Nuñez, Chazdon & Russo, 2019). In our case, the "climax system" (forest) replaces an ancient landscape with unique diversity and important ecosystem services and biodiversity (Greiner, Gordon & Cocklin, 2009). Therefore, in this case, management methods should incorporate natural disturbances (e.g. frequent and hot fires) that minimize opportunities for the environmental conditions associated with the climax system to occur. With such management interventions, savannas could be conserved, and the characteristic biodiversity of these ecosystems can be maintained.

DATA AVAILABILITY STATEMENT

The data used in this study are provided with the Supplementary information (see "Available Data.xlsx").

AUTHOR CONTRIBUTIONS

S. L. Jamison-Daniels – conceptualization, data collection, methodology, data curation, formal analysis, writing – original draft

W. D. Kissling – conceptualization, methodology, supervision, writing – review and editing

M. Botha – data collection, writing – review and editing

M. Harris – data collection, methodology, writing – review and editing

C. Gordon – methodology, writing – review and editing

M. Greve - conceptualization, data collection, funding acquisition, methodology, supervision, writing – review and editing

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