COMMUNITY ECOLOGY – ORIGINAL RESEARCH

The causes and effects of indigenous C4 grass expansion into a hyper-diverse fynbos shrubland

E. C. February1 · N. M. Munyai1·2 · C. P. Tucker1·3 · W. J. Bond1

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Abstract
The cool season rainfall at our study site should favour C3 rather than C4 grasses. There are, however, several locations where C4 grasses have become dominant, suggesting that rainfall seasonality is not a constraint on distribution. Here, we explored the limitations on C4 grass distribution in a fynbos shrubland. Using δ13C values of SOM, we determined when these grasses had established. We also looked at the role of roads as conduits for establishment and asked what impact these grasses may have on fynbos species richness. We then conducted a field experiment designed to examine the extent to which soil moisture, nutrient availability, and competition with fynbos for resources influence the establishment and growth of the grasses. Finally using aerial photography, we explored the role of changes in land use on distribution. Our results showed that the establishment is recent, that roads may be acting as conduits, and that with establishment, there is a reduction in fynbos species richness and diversity. Disturbance and removing below-ground competition for resources open the system to establishment in wetter areas. This study is the first to look at the potential for C4 grasses expanding into cool season rainfall shrublands such as in Western Australia and South Africa. What is interesting about these results is that C4 grasses can establish and dominate in a cool season rainfall regime. Rather than temperature of the growing season, it is competition for resources from fynbos that prevents these grasses from encroaching.

Keywords C4 grass · Fynbos shrubland · Community ecology · Species richness · Competition for resources · Soil moisture · Nutrient availability

Introduction
Determining the effects and consequences of the human impact on the environment is an important challenge for conservation. In the ecologically and economically important savanna biome, this focus has been on both the increase of indigenous woody species as well as that of exotic grasses (Rossiter et al. 2003; Stevens et al. 2017). These studies have demonstrated that whether native or exotic, invasive species present a serious threat to biodiversity conservation. In South Africa, there have been numerous studies looking at the drivers and consequences of an increase in native woody species (O’Connor et al. 2014; Ward 2005; Wigley et al. 2010). The very few studies that have looked at grasses have concluded that the vast majority of these are not in savanna but rather invasive C3 grasses into fynbos (Milton 2004; Visser et al. 2017).

There are two distinct regions of rainfall seasonality in South Africa, a cool season rainfall regime in the south and western part of the country and a warm season rainfall regime in the north and eastern part of the country (Schulze and Maharaj 2007). The warm season rainfall regime is dominated by C4 grasses forming grasslands and savannas. In contrast, the cool season rainfall regime is dominated by shrubland vegetation and is synchronous with the Greater Cape Floristic Region (GCFR), well known for both species...
diversity (11,423 documented vascular plants) and endemism (77.9%) (Colville et al. 2015). There has been much discussion in the literature as to the consequences of woody plant encroachment in C₄ grassy ecosystems (O’Connor et al. 2014; Parr et al. 2014). There has also been some research on the expansion of C₄ grasses into arid shrublands (Masubelele et al. 2014). There has been very little discussion of either the potential for, or consequences of, C₄ grass expansion into Mediterranean climate shrublands such as that of the GCFR (Cione et al. 2002; Cowling 1983; Milton 2004). While clumps of C₃ and C₄ grass species occur in the GCFR, these grasses are not dominant, but form part of the herbaceous understory of the dominant shrubs (Cowling 1983). However, it has been suggested that the impacts of invasive C₃ grasses on fynbos biodiversity in the GCFR are considerable, but there is, as yet, very little evidence to show this (Visser et al. 2017).

In the south western region of South Africa, models forecasting climate change suggest both a decrease in cool season rainfall as well as a shift in rainfall towards the warmer months with increases in early and late season precipitation (Hewitson and Crane 2006; MacKellar et al. 2014). An analysis of temperature records also shows that temperatures have increased for the region (MacKellar et al. 2014). With the availability of earlier and later rainfall and an increase in temperature, it is possible that the nitrogen and water-use efficient C₄ grasses may increase in dominance (Ehleringer et al. 1997). These grasses cure rapidly in the dry season with the potential to change both the fire regime and the intensity of fires (Bond 2008; D’Antonio and Vitousek 1992). An increase in the dominance of C₄ grasses may not only affect the fire regime, but may also result in decreased species richness and diversity or even a biome shift from shrubland to grassland (Milton 2004). It is, therefore, extremely important to understand the determinants for C₄ grass distribution in fynbos and the extent to which management intervention may be necessary.

To help understand the potential for C₄ grasses to expand into fynbos, we explore the constraints on C₄ grasses growing in a fynbos-dominated area. At our study site in the Potberg section of the De Hoop Nature Reserve in the Western Cape Province of South Africa, there are several patches of indigenous C₄ grasses that have replaced the dominant shrubs (Fig. 1, Supp. Fig. 1). These patches are an anomaly given differences in the physiology of C₃ and C₄ grasses that are thought to influence distribution (Ehleringer et al. 1997). Here, we ask if these observed patches of C₄ grass are a natural part of the system, or representative of a recent expansion. We also ask, if the patches are more recent, then are roads acting as conduits for these grasses to establish or are the patches relics from when the reserve was still a commercial farm. We then ask if the grasses have any effect on fynbos species richness. Finally, we carried out an experiment in the field designed to examine how C₄ grasses may establish and persist in Mediterranean climate shrublands such as fynbos.

There have been numerous publications documenting the invasion of Californian shrublands by African grasses (Cione et al. 2002; Hattersley 1983; Lambrinos 2000). There have, however, been no studies either in South Africa or Western Australia documenting the colonisation of shrubland by native C₄ grasses. This is despite both Australian and South African shrublands bordering on Savanna. With global climate change, it is imperative for the development of an understanding as to how these boundaries are maintained and what the Anthropogenic effect on them may be.

### Methods

#### Study site

The study was located at the Potberg section (S34.3750° E20.5336°) of the De Hoop Nature Reserve 47 km from the town of Bredasdorp in the Western Cape Province of South Africa. The soils over the eastern valley section of Potberg where the study was conducted are extremely infertile podsoils derived from aeolian sands with seasonal waterlogging common on the lower slopes (Fey 2010).

The vegetation at our study site is classified by Mucina and Rutherford (2006) as Agulhas Sand Fynbos and Alber-tinia Sand Fynbos with shrubs including Protea repens, Protea nerifolia, Erica quadrangularis, and Leucadendron lauren-lorum. Major species of Restionaceae include Ischyrolepis capensis, Staberoha banksii, and Hypodiscus aris-tatus. The patches of C₄ grasses are dominated by Imperata cylindrica, Eragrostis curvula, Setaria sphacelata var. tarta, and Cynodon dactylon.

Mean annual rainfall for De Hoop Nature Reserve is 572 mm (CapeNature 2016). The wettest months are from June to August (12% of MAP per month), and the driest months are from December to February (4–5.5% of MAP per month). August is the coldest month (max 20 °C, min 10 °C) and February is the warmest month (max = 24 °C, min = 20 °C) (CapeNature 2016).

#### Soil carbon isotopes

We used differences in carbon isotope ratio of the grasses and shrubs at our study site to make a determination as to how recently the C₄ grass patches had established (Connin et al. 1997; West et al. 2000). In July 2016, we sampled at eight locations south of and along the access road into the reserve between S34.40694° E20.56388° and S34.42166° E20.63138° (Fig. 2, Supp. Fig. 2). At each of the eight locations, we sampled soil from the sidewall of a pit dug in a...
C₄ grass patch as well as in the fynbos not more than 50 m away. These soils were sampled at three depths, 0–5, 5–10, and 10–20 cm. At each location, we also sampled the leaves from the three most dominant fynbos and two most dominant grass species. We only report on the 0–20 cm soil horizon (surface soils) because of unrelated fractionation processes at 50 cm depth causing enrichment of soil δ¹³C unrelated to the inputs from C₃- or C₄-derived carbon (Balesdent and Mariotti 1996).

Prior to stable isotope analysis, all leaf and soil samples were dried to a constant weight at 70 °C and all leaf samples for each location were pooled before grinding to a fine powder using a Retsch MM 200 ball mill (Retsch, Haan, Germany). We determined δ¹³C values for all samples using a Flash 2000 organic elemental analyser coupled to a Delta V Plus isotope ratio mass spectrometer via a Conflo IV gas control unit (Thermo Scientific, Bremen, Germany). Precision of duplicate analysis is 0.1 ‰ (February et al. 2011).

Roads as conduits for grass establishment

Roads are considered as one of the anthropogenic corridors that may facilitate the spread of species beyond their native habitat and geographical boundaries (Gelbard and Belnap 2003; Rahlao et al. 2010). To determine the influence of the access road in the reserve on the spread of C₄ grasses, we established a series of 5 × 10 m plots located 5 m in from the very edge of the road (the Road verge plots). We also established a series of 10 × 10 m plots perpendicular to the road and 100 m from the edge of, and parallel to, the Road.
verge plots (the Interior plots). We did this at 500 m intervals on both sides of the road for 12.5 km from S34.3753° E20.5263° to S34.4102° E20.5791° in July 2014 (Supp. Fig. 2). A total of 100 plots were sampled of which 50 were Road verge plots and 50 Interior plots. On each plot, all the grasses were identified to species level and total grass canopy cover recorded as a percentage of the total vegetation cover (Cowling 1983; Hanley 1978). This was made under the assumption that grass and fynbos species in the plots combined to make up 100% canopy cover. We expressed the cover of the C₃ and C₄ grasses as a proportion of the total grass cover. Photosynthetic pathway of the grasses is according to (Bentley and O’Connor 2018; Bouchenak-Khelladi et al. 2014; Chatanga and Sieben 2019; Hattersley 1983; Ripley et al. 2015; Vogel 1978). Each plot was georeferenced using a Garmin eTrex 10 GPS (Garmin, United States of America).

**C₄ grass and fynbos species richness**

While no studies have identified the potential effect that indigenous C₄ grass expansion may have on species richness of Mediterranean shrublands, several studies have identified the impact of invasive grass on species richness and fire intensity (D’Antonio and Vitousek 1992; Franklin et al. 2006; Lambrinos 2000). To determine the impact of indigenous C₄ grasses on fynbos species richness, we identified all plant species in a total of one hundred 10 m × 5 m plots, 50 of which were classified as grass-dominated (Grass plots) and 50 fynbos-dominated (Fynbos plots) (Supp. Fig. 2). Plot selection was based on the visual perception of the percent canopy cover, where more than 60% grass was classified as grass-dominated, with the same for fynbos. The plots were spaced approximately 50 m apart and specifically selected based on the

![Orthorectified aerial photographs of the study site from 1954 to 2020 illustrating the extent of the farming activities through time. Black dots are the locations where we sampled for our study. The property was taken over by the conservation agency Cape Nature in 1977.](image)
dominant vegetation using an estimation of cover abundance as described above (Hanley 1978; Mueller-Dombois and Ellenberg 1974). These plots were also specifically chosen to be away from the disturbance created by the road. In the field, voucher specimens were taken, and species identifications confirmed by cross referencing with specimens housed in the Bolus Herbarium at the University of Cape Town.

**Factors influencing grass establishment and persistence**

The growing season for the C₄ grass species at Potberg is during the warmer months of the year characteristic of the tropical grassland and savanna biomes (Vogel 1978). Here, we describe an experiment designed to examine the extent to which topographic position (a surrogate for soil moisture availability), nutrient availability, and competition with fynbos for resources influence the establishment and growth of C₄ grasses at our study site (Supp. Fig. 2). The experiment was set up at the end of May 2015 along a catenial sequence in fynbos where there were no C₄ grasses. Plots were laid out at three positions along the gradient, bottom, middle, and top. A soil auger was used to explore presence and depth to water table at each plot site. Water tables were present within 80 cm of the soil surface at the bottom of the slope, at 1.2–1.5 m mid-slope, and with no water table to at least a depth of 2 m at the top of the slope.

At each topographic position along the slope, we transplanted a tuft of grass collected in June 2015 from a single patch of Setaria sphacelata var. torta into one of four (20 × 20 cm) treatments: trenched and not-trenched both with and without fertiliser. Each group of four treatments was replicated five times at three locations at each topographic position with each replicate group of treatments ~ 100 m apart from the next. This summed to a total of 180 transplants (3 topographic positions × 3 locations at each position × 4 treatments × 5 replicates per treatment). We restricted root competition between the grass and the surrounding fynbos species by vertically inserting a 20 cm × 20 cm plastic sheet into the ground, on four sides of the targeted grass, with 1 cm of plastic protruding above the soil surface. We also applied, where relevant, 60 g of a slow-release granular fertiliser encapsulated in a multilayer polymeric coating, Multicote 4* urea-N (42-0-0, N:P:K, Haifa Group, South Africa). A slow-release fertiliser was specifically used to prevent rapid volatilization of the nitrogen (Matimati et al. 2014).

The experiment was finally terminated in May 2017 (after 2 years) when the surviving grasses were harvested and oven-dried at 70 °C for 48 h before weighing to determine biomass.

**Land-use history**

Using repeat aerial photographs from 1954 to 2020, we explored whether changes in land use may have influenced the introduction of C₄ grasses at our study site. For this purpose, aerial photographs of the study site were sourced from National Geo-spatial Information in Mowbray, Cape Town. Using ArcGIS software, we then georeferenced the images relative to ESRI’s world imagery base map (2020). Finally, we inserted the locations where we had sampled C₄ grasses onto these georeferenced images to allow for visual inspection to ascertain any changes in land use through time (Fig. 2).

**Statistics**

**Soil carbon isotopes**

The stable carbon isotope data were log-transformed after which Student’s t tests were implemented in base R and the differences between fynbos and grass sites calculated separately for leaf, litter, and soil samples (R_Development_Core_Team 2019). Using a simple mixing model based on the δ¹³C values of the soil and end-member (mean) δ¹³C values of the leaves of the grasses and fynbos, we determined the relative proportion of C₃ (fynbos) and C₄ (grasses)-derived carbon in the soil with depth (February et al. 2013; Mordelet et al. 1997). We also added 1‰ to the δ¹³C values of the soil at − 5 cm and 2‰ to the − 10 and − 20 cm layers to account for the increase in fractionation with depth (Boström et al. 2007; Ehleringer et al. 2000). This mixing model was only applied to the 0–20 cm soil horizon (surface soils) because of fractionation processes at 50 cm depth causing enrichment of soil δ¹³C unrelated to the inputs from C₃ or C₄-derived carbon (Balesdent and Mariotti 1996).

**Roads as conduits for grass establishment/C₄ grass and fynbos species richness**

Differences in average canopy cover for the grasses between the Road verge and Interior plots were determined using Student’s t tests. To quantify plant species diversity between the Road verge and Interior plots as well as Grass and Fynbos plots, we use the Shannon diversity index (H′) as a measure of species abundance and richness. We calculated the relative abundance of each species, in each of the two communities, as the frequency of an individual species relative to the total number of all species in a community. Species evenness (equitability) was calculated as:

\[ E_H = H / \ln S. \]
The number of species in a community with equal abundances, effective number of species ($D$), (Jost 2006) was calculated as:

$$D = \exp \left( - \sum_{i=1}^{S} Pi \ln Pi \right) = \exp (H)$$

Where: $H' = \text{Shannon diversity index}$, $S = \text{total number of species in a community}$, and $P_i = \text{the proportion of individuals in the } i\text{th species}$ (Jost 2006; Tramer 1969).

The similarity in species composition between the Road verge and Interior plots as well as Grass and Fynbos plots was determined using Sorensen similarity index.

**Factors influencing grass establishment and persistence**

For the transplant experiment, we counted how many individuals had died at the end of the experiment in each treatment and at each slope position. These data were then used to calculate a percentage survival for each treatment at each slope position for the three slopes. A generalised linear mixed-effects model (GLM) was then used to test the effect of treatment, slope position and the interaction between these:

$$(P.\text{Survival} \sim \text{SlopePosition} \times \text{Treatment} + \text{Error(Slope)})$$

We performed a GLM analysis of the effect of treatment, slope position, and the interaction between these on total plant biomass:

$$(\text{TotalBiomass}(\log) \sim \text{SlopePosition} \times \text{Trenching} \times \text{Fertilizer})$$

The GLM used bottom, no trench, and no fertiliser as baselines for the predictors. All dead individuals were removed from the model in this analysis resulting in a total of 140 individual grasses with sample sizes per treatment ranging from 8 to 14.

Both GLM’s were run in R. Significant differences were assessed using p values generated using Satterthwaite’s degrees of freedom method as implemented in the lmerTest package (Kuznetsova et al. 2017).

**Results**

**Soil carbon isotopes**

The end-member $\delta^{13}C$ values for the grasses ($-16.26 \%e \pm 1.55 \text{ Stderr}$) at our study site were significantly different from the fynbos ($-27.9 \%e \pm 0.44 \text{ stdderr}$, $P < 0.001$). The $\delta^{13}C$ values for the litter at the surface varied considerably on the grass patches ranging from $-16.5\%e$ to $-28.3\%e$ ($-25.0\%e \pm 1.51 \text{ stdderr}$). These values were, however, not significantly different from the fynbos litter (mean $\delta^{13}C = -27.7\%e \pm 0.26 \text{ stdderr}$). While $\delta^{13}C$ values for the soils for the three depths are significantly different ($P < 0.001$) for the two communities, these values are more like fynbos ($-27.9 \%e$) than $C_4$ grass ($-16.26 \%e$, Fig. 3). The results of our mixing model for the fynbos plots show 98% fynbos in the leaf litter and 97% fynbos through the soil profile down to $-20 \text{ cm}$. In the grass plots, values range between 25% grass in the litter and 20–23% $C_4$ grass down to $-20 \text{ cm}$.

**Roads as conduits for grass establishment**

Average canopy cover for grasses on the Road verge plots was significantly higher 23.6% ($\pm 3.7 \text{ std err}$) than in the Adjacent plots 12.8% ($\pm 3.1 \text{ std err}$, $P < 0.01$). A Sorensen similarity index shows that there is an 81% similarity in grass species composition between the Road verge and Interior plots with 18 grass species in the Road verge plots and 14 in the Interior plots (Fig. 4). Despite this high percentage of species in common (13), a Shannon index of diversity shows that the Interior plots had higher grass species diversity (2.56) than the Road verge plots (1.50) with a higher number of species with equal abundance (effective number of species, 12.9 and 4.5, respectively). Grass species evenness was also higher on the Interior plots (0.97) than the Road verge plots (0.57). In an analysis of the relative abundance (proportional frequency) of each species, over 80% of the grasses in the Road verge plots belonged to three species, *Cynodon dactylon*, *Eragrostis curvula*, and *Tribolium uniolae* (Table 1). The three most common grass species in the Interior plots, *Pennisetum thunbergii*, *Aristida diffusa*, *
and *Cynodon dactylon* only make up 32% of all the grass species (Table 1).

### C₄ grass and fynbos species richness

Average canopy cover for grasses on the Grass plots was 83.5% (± 1.9 std err) and that for fynbos on the Fynbos plots was 93.6% (± 1.6 std err). A Sorensen similarity index shows that there is a 47% similarity between the Fynbos and Grass plots with 27 species in common (Table 2). The Fynbos plots had a higher total count of species (73), higher Shannon index of diversity (3.86), and higher effective number of species (48) than the Grass plots (Total count 41, Shannon diversity index 3.48, and effective number of species 32). In

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**Table 1** The relative abundance (%) and photosynthetic pathway (Phot.) of all the grasses in our study of roads as conduits for grass invasion

| Road-side plots | Species          | %     | Phot. | Interior plots | Species                  | %     | Phot. |
|-----------------|------------------|-------|-------|----------------|---------------------------|-------|-------|
| 1               | *Cynodon dactylon* | 25.5  | C₄    | *Pennisetum thunbergii* | 12.0 | C₄    |
| 2               | *Eragrostis curvula* | 21.6  | C₄    | *Aristida diffusa* | 10.0 | C₄    |
| 3               | *Tribolium uniolae* | 18.6  | C₃    | *Cynodon dactylon* | 10.0 | C₄    |
| 4               | *Tenaxia disticha* | 7.8   | C₃    | *Tenaxia disticha* | 10.0 | C₃    |
| 5               | *Aristida diffusa* | 5.9   | C₄    | *Themeda triandra* | 10.0 | C₄    |
| 6               | *Pennisetum thunbergii* | 3.9 | C₃    | *Trichogonion uniolae* | 10.0 | C₃    |
| 7               | *Pentameris aroides* | 2.9   | C₃    | *Cortaderia selloana* | 6.0  | C₃    |
| 8               | *Briza maxima* | 2.0   | C₃    | *Eragrostis capensis* | 6.0  | C₄    |
| 9               | *Enneapogon desvauxii* | 2.0   | C₄    | *Pentameris aroides* | 6.0  | C₃    |
| 10              | *Stenotaphrum secundatum* | 2.0   | C₄    | *Andropogon eximius* | 4.0  | C₄    |
| 11              | *Andropogon eximius* | 1.0   | C₄    | *Eragrostis curvula* | 4.0  | C₄    |
| 12              | *Eragrostis capensis* | 1.0   | C₄    | *Fingerhuthia africana* | 4.0  | C₄    |
| 13              | *Fingerhuthia africana* | 1.0   | C₄    | *Pentameris pallida* | 4.0  | C₃    |
| 14              | *Milanis repens* | 1.0   | C₄    | *Stenotaphrum secundatum* | 4.0  | C₄    |
| 15              | *Pennisetum clandestinum* | 1.0   | C₄    |                      |          |       |
| 16              | *Pentameris pallida* | 1.0   | C₃    |                      |          |       |
| 17              | *Phalaris Minor* | 1.0   | C₃    |                      |          |       |
| 18              | *Themeda triandra* | 1.0   | C₄    |                      |          |       |
### Table 2

The relative abundance (%) of all species as well as photosynthetic pathway of the grasses in our study of the effects of C4 grass on fynbos species richness.

| Fynbos Plots | Species                  | % Phot. | Grass Plots | Species                  | % Phot. | Phot. |
|--------------|--------------------------|---------|-------------|--------------------------|---------|------|
| 1            | Cliffortia stricta       | 6.27    |             | Cynodon dactylon         | 6.16    | C4   |
| 2            | Leucadendron salignum    | 5.01    |             | Merxmuella disticha      | 4.79    | C3   |
| 3            | Passerina rigida         | 4.76    |             | Setaria sphacelata      | 4.79    | C4   |
| 4            | Phylica purpurea         | 4.76    |             | Helichrysum Patulum     | 4.45    |      |
| 5            | Erica colorans           | 4.51    |             | Cliffortia stricta      | 4.11    |      |
| 6            | Serraria fasciflora      | 4.51    |             | Leucadendron salignum   | 4.11    |      |
| 7            | Stoebe plumosa           | 4.01    |             | Trichocephalus stipularis| 4.11    |      |
| 8            | Trichocephalus stipularis| 4.01    |             | Cortaderia seloana      | 4.11    | C3   |
| 9            | Searsia glauca           | 3.51    |             | Eragrostis curvula      | 4.11    | C4   |
| 10           | Morela quercifolia       | 3.26    |             | Notobubon galbanum      | 3.77    |      |
| 11           | Leucadendron coniferum   | 2.76    |             | Melica decumbens        | 3.77    |      |
| 12           | Erica bodkinii           | 2.51    |             | Berzilia lanuginosa     | 3.42    |      |
| 13           | Erica plukenetii         | 2.26    |             | Searsia glauca          | 3.42    |      |
| 14           | Helichrysum patulum      | 2.26    |             | Aristida diffusa        | 3.42    | C4   |
| 15           | Peucedanum galbanum      | 2.26    |             | Eustachys paspaloides   | 3.42    | C4   |
| 16           | Protea repens            | 2.26    |             | Morela quercifolia      | 3.42    |      |
| 17           | Berzelia abrotanoides    | 1.50    |             | Helichrysum teretifolium| 3.08    |      |
| 18           | Gnidia tenella           | 1.50    |             | Themeda triandra        | 3.08    | C4   |
| 19           | Leucadendron truncatula  | 1.50    |             | Stoebe Plumosa          | 2.74    |      |
| 20           | Penaea mucronata         | 1.50    |             | Passerina rigidia       | 2.74    |      |
| 21           | Agathosma ciliaris       | 1.25    |             | Erica rhapalantha      | 2.40    |      |
| 22           | Berzelia lanuginosa      | 1.25    |             | Pennisetum thunbergii   | 2.40    | C4   |
| 23           | Carpobrotus edulis       | 1.25    |             | Dodonaea angustifolia   | 2.05    |      |
| 24           | Chironia baccifera       | 1.25    |             | Myrsine africana        | 1.71    |      |
| 25           | Erica discolor           | 1.25    |             | Phylica purpurea        | 1.71    |      |
| 26           | Protea nerifolia         | 1.25    |             | Sporobolus africanus    | 1.71    | C4   |
| 27           | Aristida diffusa         | 1.25    |             | Carpobrotus edulis      | 1.71    |      |
| 28           | Eragrostis curvula       | 1.25    | C4          | Chironia baccifera      | 1.37    |      |
| 29           | Pentameris pallida       | 1.25    | C4          | Imperata cylindrica     | 1.03    | C4   |
| 30           | Merxmuella disticha      | 1.25    | C4          | Pentaschistes aroides   | 1.03    | C3   |
| 31           | Tribolium uniolae        | 1.25    | C3          | Serraria fasciflora     | 0.68    |      |
| 32           | Erica ampuscata          | 1.00    | C3          | Leucadendron linifolium | 0.68    |      |
| 33           | Erica cafra              | 1.00    |             | Sideroxylon inerme      | 0.68    |      |
| 34           | Helichrysum teretifolium | 1.00    |             | Thamnochorus cinereus   | 0.68    |      |
| 35           | Leucadendron linifolium  | 1.00    |             | Staberoha banksii       | 0.68    |      |
| 36           | Myrsine africana         | 1.00    |             | Osyris speciosa         | 0.68    |      |
| 37           | Protea susannae          | 1.00    |             | Acacia mearnsii         | 0.34    |      |
| 38           | Pentameris aroides       | 1.00    |             | Searsia undulata        | 0.34    |      |
| 39           | Pennisetum thunbergii    | 1.00    | C3          | Ehrharta calycina       | 0.34    | C3   |
| 40           | Erica rhapalantha       | 0.75    | C4          | Erica colorans          | 0.34    |      |
| 41           | Lachnaea axillaris       | 0.75    |             | Tribolium uniolae       | 0.34    | C3   |
| 42           | Cynodon dactylon         | 0.75    |             |                      |         |      |
| 43           | Themeda triandra         | 0.75    | C4          |                      |         |      |
| 44           | Adenandra gummifera      | 0.50    |             |                      |         | C4   |
| 45           | Agathosma cerefolium     | 0.50    |             |                      |         |      |
| 46           | Erica seriphifolia       | 0.50    |             |                      |         |      |
| 47           | Leucadendron meridianum  | 0.50    |             |                      |         |      |
| 48           | Leucospermum trican      | 0.50    |             |                      |         |      |
| 49           | Metalasia densa          | 0.50    |             |                      |         |      |
an analysis of the relative abundance of each species within each community, 80% of the species richness is made up by 32 species on the Fynbos plots and 20 species on the Grass plots (Table 2).

Factors influencing grass establishment and persistence

A wildfire burned through the entire experiment in the first week of September 2015, 3 months after the start of the experiment. Six plants had died before the fire and a further 36 plants did not recover from the fire; however, only 3 of the transplants died in the post burn environment. At the end of the experiment, 19 months after the fire, significantly ($\beta = 0.82, t = -2.00, P < 0.05$) more grass transplants had died at the top of the slope (19) than in the middle (13) and bottom (8). There was no significant difference in mortality between the treatments. The interaction effects on biomass using a GLM excluding dead individuals show a significant interaction between trenching and fertiliser (Fig. 3). Although the bottom of the slope had higher total above ground biomass with the lowest number of dead individuals, this difference was not statistically significant when looking at slope position alone.

The analysis shows a significant increase in biomass with the addition of fertiliser ($\beta = 0.89, t = 4.69, P < 0.001$) and a further significant increase with trenching and fertiliser combined ($\beta = 0.64, t = 2.39, P = 0.02$), Fig. 3.

Land-use history

Prior to incorporation into the De Hoop Reserve in 1977, Potberg was a privately owned farm. The aerial photographs from 1954 to 1981 are not very good quality (Fig. 2). These photographs do, however, show both crop and pasture with the crops being on the north side of the road and the pasture on the south side where we sampled and where we find C4 grass patches. The aerial photographs also show the enormous amount of disturbance across the entire study site where the white underlying sand has been exposed possibly related to an increase in fire frequency with farming (Fig. 2). The 2015 image taken just before the fire shows what the vegetation should look like without disturbance. In contrast, the 2020 image shows some white sand still evident after the 2015 fire because of the slow recovery of the vegetation possibly related to the 2015-2017 drought (Otto et al. 2018).
Discussion

Several studies have demonstrated an anthropogenically induced increase in woody cover in grassy ecosystems (Lohmann et al. 2014; Tews et al. 2006; Van Auken 2000). There have, however, been very few studies documenting a recent increase in indigenous grasses with a decrease in shrubs (Hoffman and Cowling 1990; Masubelele et al. 2014). Our results show the recent origin of the grasslands at our study site. Relative to the fynbos plots, the SOM δ¹³C values for the grass plots are significantly more enriched, demonstrating the influence of the grass roots in these plots. The δ¹³C values in these plots are, however, closer to the end-member values for fynbos (− 27.9‰) than grasses (− 16.26‰) demonstrating the more recent anthropogenic origin of the patches. The end-member δ¹³C values for the grasses and shrubs at our study site are sufficiently different for us to reliably use a mixing model to ascertain whether the grass patches at our study site are of recent origin. The results of our mixing model confirm the recent anthropogenic origin of the grass patches, indicating that only around 20% of the soil carbon in the grass plots may be attributed to grasses.

If the grass patches are recent, then we considered if roads were acting as conduits for the distribution of grasses into the ecosystem. It has been shown that, in several national parks in North America, there is a high incidence of invasive grasses along road verges declining with distance from the road (Gelbard and Belnap 2003). Our results also show a greater diversity of grass species and lower species evenness along the road verge than further inland. Except for Cortaderia selloana (fountain grass), the South American C₃ grass, all the grasses in both the Interior and Road verge plots, are indigenous grasses that may naturally occur in the area. Of the top three species in both communities, the most prolific grass in the Interior plots, Pennisetum thunbergii, prefers undisturbed vegetation while the other five are all classified as weeds or are known to occupy disturbed environments (Fish et al. 2015; van Oudtshoorn 2002). The high number of weedy C₄ species in the Interior plots and the degree of similarity between the Road verge and Interior plots suggest that the recent origin of the grassy patches at our study site may be from the road into the reserve. We speculate that seeds probably lodge in vehicles and are then distributed into the disturbed area along road verges that have the ideal conditions for C₄ grasses, including high light, increased available water, and increased nutrients (Gelbard and Belnap 2003; van Oudtshoorn 2002).

Alien invasive plants have been identified as the second most important threat to biodiversity after habitat loss (Foxcroft and Richardson 2003). Of these plants, grasses are particularly destructive ecosystem engineers, capable of changing the functioning of an ecosystem by impacting on both relative abundance and species diversity (Hobbs and Huenneke 1992). Fynbos species diversity and endemism are among the highest for any region of similar size worldwide and the most ecologically threatened of all plant kingdoms (Colville et al. 2015; Manning and Goldblatt 2012). It is, therefore, important to understand the potential effect that C₄ grass colonisation may have on fynbos species diversity. Our results show that, when they become dominant, grasses have a significant impact on fynbos species diversity, suggesting that any increase in C₄ grasses will result in a negative effect on species diversity. The most extreme effect is a biome switch from fynbos shrublands to a grassland.

Several studies have suggested that the high species diversity in fynbos is only possible, because there is no competition for resources with different species segregating along several niche axes including light, nutrients, and water (Araya et al. 2011; Richards et al. 1997). With the high diversity of fynbos species occupying all available niche axes, we designed an experiment to examine the extent to which topographic position (moisture availability), an increase in nutrients and a decrease in competition for resources may influence the establishment and growth of C₄ grasses. The results of this experiment show a linear increase in grass mortality with slope position with the highest mortality at the top of the slope where available water is lowest. There was, however, no significant effect of nutrients or fynbos competition on grass mortality.

As regards subsequent grass growth, our results are surprising. Topographic position has a strong influence on both soil moisture and nutrient availability, because riparian zones lie at the interface between terrestrial and aquatic ecosystems, collecting both surface and subsurface runoff containing a high percentage of nutrients (Jacobs et al. 2007; Knapp et al. 1993). It is soil moisture, however, that has also been identified as the most likely driver of the differences in grass biomass along topographic gradients in North American prairies (Knapp et al. 1993). Our results demonstrate the expected effect of soil moisture on grass growth. They also show the expected effect of greater growth in the nutrient addition treatments. What we had not expected was the growth response to an increase in nutrients and lack of competition for resources as exemplified by the trenching and nutrient addition treatments in our experiment. Neither ‘climate’ nor nutrient-poor podzolic soils excluded C₄ grasses in our study, but below-ground competition from intact fynbos, working with climate and soils, acted as a significant deterrent to grass colonisation. These results would suggest that C₄ grasses would be favoured with any anthropogenic change, resulting in destruction of fynbos and their root systems.
by an increase in disturbance along with an increase in available moisture and nutrients.

While our results do suggest that roads may act as conduits for the establishment of fynbos by C_4 grasses, these results also demonstrate that the ecological switch from scattered clumps of C_4 grasses to a grassland is made more difficult by competition for resources with fynbos. We looked at a series of aerial photographs taken between 1954 and 2020 to ascertain the level of disturbance at our plots through time. While the quality of these photographs is poor, it is possible to see a high degree of farming activity from ploughing to paddocks on all our plots. Some of the grasses certainly came in along roads, but we speculate that the main patches of C_4 grass that we see today may originate from disturbance of the fynbos and an increase of nutrients with farming allowing for the ecological switch from scattered clumps to extensive grassland patches, especially on lower slopes slope where moisture is highest.

Model predictions for our study area suggest a shift in rainfall towards the warmer months with increases in early and late season precipitation (Hewitson and Crane 2006; MacKellar et al. 2014). Along with predicted increases in temperature, the availability of earlier and later rainfall may allow for the nitrogen and water-use efficient C_4 grasses to increase in dominance (Ehleringer et al. 1997). We speculate that such an increase is only likely with disturbance of fynbos such as is suggested in this study.

Conclusion

Our results show that roads may indeed act as conduits for the establishment of C_4 grasses in fynbos, and that any increase in the biomass of grasses will result in a decline in plant species richness and diversity. These results also show that with increasing amounts of fertiliser and water, the biomass of C_4 grasses will increase. It is, however, reasonably well known that farmers increase the biomass of their crops by adding fertiliser and water. What is interesting about these results is that C_4 grasses can establish and grow in the cool season rainfall regime of the south western Cape. Rather than temperature of the growing season, it is competition for resources from fynbos that prevents these grasses from encroaching. The inference is that seriously disturbing fynbos and removing below-ground competition for resources, opens the system to establishment by C_4 grasses. Fynbos is not the only species rich Mediterranean shrubland bordered by C_4 grasses, as a similar system can be found in south western Australia (Beard et al. 2000). We need to be putting more emphasis on understanding the boundaries between grassy and shrubby biomes in the southern continents since, should the grassy ecosystems expand, the impact on the species rich heathland floras would be devastating. Our study is but a first glimpse with much more work needed.

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Author contribution statement ECF, NMM, and WJB conceived and designed the experiments. All authors performed the experiments. ECF and NMM analysed the data and wrote the first draft of the manuscript. All authors contributed to subsequent drafts and provided editorial advice.

Data availability statement The data used in this study are available on the University of Cape Town open access database at https://doi.org/10.25375/uct.12647057.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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