CHAPTER 2.

SPATIAL AND HABITAT ATTRIBUTES OF EXPERIMENTAL STUDY PLOTS

2.1 INTRODUCTION

There are a variety of approaches researchers employ to investigate whether or not density effects are operating in plant populations. These may include experimental manipulations whereby individual plants are manually positioned in artificial arrays (Kunin 1997a; Knight 2003) alternatively, researchers may utilise a range of densities that occur naturally within a given study system (e.g. Kunin 1992; Roll *et al.* 1997; Steven *et al.* 2003; Kirchner *et al.* 2005), manipulate plants in natural systems to conform with predetermined densities (e.g. Schmitt 1983) or utilise both natural and experimental populations in combination (e.g. Van Treuren *et al.* 1993; Van Treuren *et al.* 1994). Regardless of whether the study system involves artificial or natural plant assemblages, the variable "density" can be approached in a number of ways. As pointed out in Chapter 1, density (not to be confused with population size) is defined as the number of individuals per unit area (Kunin 1997a). This is a measure of absolute density, but density can also be a relative measure i.e. "sparse" versus "dense" and it is not uncommon for researchers to investigate density effects using both absolute and relative density measures (e.g. Roll *et al.* 1997). Needless to say, including non-reproductive individuals in density calculations can confound the relationship between density and reproductive output and measurements should only consider the number of "reproductive individuals" per unit area.

Measures of both absolute and relative density were utilised in this study (as described below). Absolute density was quantified at the plot level (number of reproductive individuals per m^2) and at the focal plant level (measurement of near neighbour distances-NND); the relative approach of "sparse" versus "dense" (few versus many reproductive individuals) was also included. In addition, several habitat attributes were assessed to gain a broad idea of the overall properties of the study sites and the immediate local environment within study plots. This was warranted because the study was undertaken in a natural system and similarity among sites was desirable. Furthermore, characteristics at the patch level may be important in shaping density and reproduction i.e. it is possible that differences in some attributes may reflect the quality of the area under study. For example, higher species richness and taller growth may be indicative of greater within patch resource availability, which may provision resources for increased reproductive output.

2.2 AIMS

This purpose of this chapter is to provide an overview of the methods that were employed to establish the experimental plots utilised for field data collection. It also details the pertinent density attributes (absolute and relative) of the plots, which were subjected to statistical analysis to confirm that their spatial properties were suitable for the purposes of the study. A simple assessment of some basic habitat attributes (e.g. species richness) for each study species at each site and between densities within sites was undertaken to see if there were any obvious differences that may substantially influence results.

2.3 METHODS

2.3.1 Experimental Plot Establishment

For each study species at each site, a reconnaissance was undertaken on foot to gain an overview of the population's general layout and limits, and to identify potential focal plants around which plots could be established. Focal plants (FPs) provided a central point for plot set up. Focal plants were reproductive, healthy individuals of similar size. For each species, five dense and five sparse plots were established at each site. The allocation of a plot to either the sparse or dense category was determined by estimating the number of reproductive individuals present within the plot area and the distance between the focal plant and its nearest five reproductive neighbours. These variables were analysed to ensure that there were significant differences between sparse plots and dense plots (see results below) i.e. that there were differences in relative density between the plot types.

Once focal plants were allocated for *T. australe* and *W. luteola*, a 5m x 5m ($25m^2$) quadrat was laid down with the FP at the centre (Figure 2.1a). Since *D. sieberi* is a woody shrub and larger than the herbaceous *T. australe* and *W. luteola*, plots were increased to 10 x 10m ($100m^2$) for this species. The quadrat was constructed from PVC electrical conduit (20mm diameter). To facilitate the counting and mapping of reproductive individuals and assessment of habitat attributes within plots, the quadrat was further divided into 1x1m squares using elasticised rope attached at 1m intervals (Figure 2.1c). *Dillwynia sieberi* plots were delineated by laying out the 5m x 5m quadrat four times around the centrally positioned focal plant (Figure 2.1b). The FPs were then labeled using PermaTags™ and wooden stakes or tent pegs were used to semi-permanently mark the corners of the plot; the quadrat was then removed. Once the quadrat was in position, the distance from the FP to its five nearest reproductive neighbours was measured, the mean of this giving a measurement of absolute density (mean near neighbour distance (NND)) for the focal plant. These five near neighbours were then mapped onto a data sheet. In addition, all reproductive individuals were mapped and later counted, providing a second measure of absolute density. This map also provided baseline data so that any changes occurring within plots between seasons could be tracked.

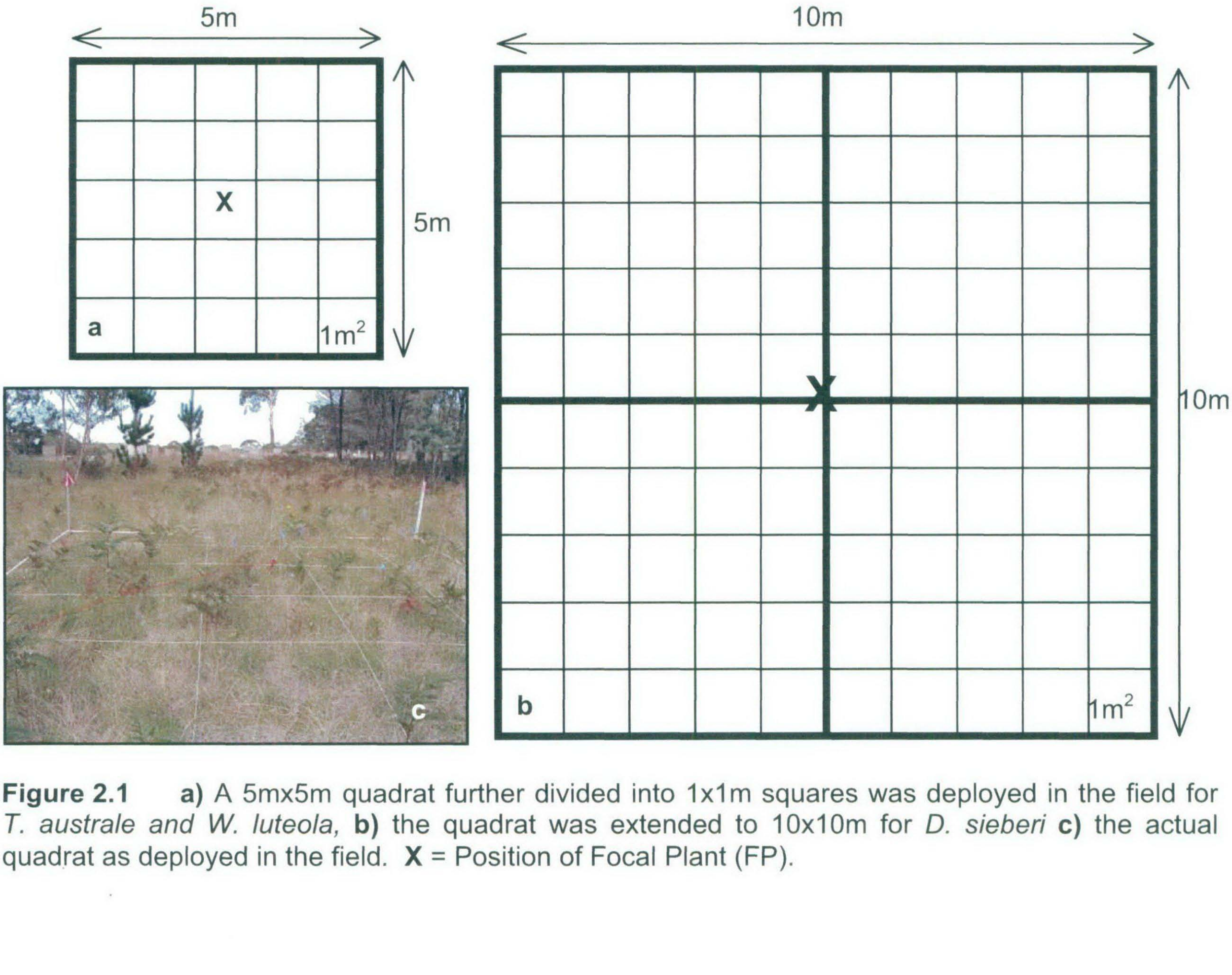

Figure 2.1 **a)** A 5mx5m quadrat further divided into 1x1m squares was deployed in the field for *T. australe and W. luteola,* **b)** the quadrat was extended to 10x10m for *D. sieberi* **c)** the actual quadrat as deployed in the field. **X** = Position of Focal Plant (FP).

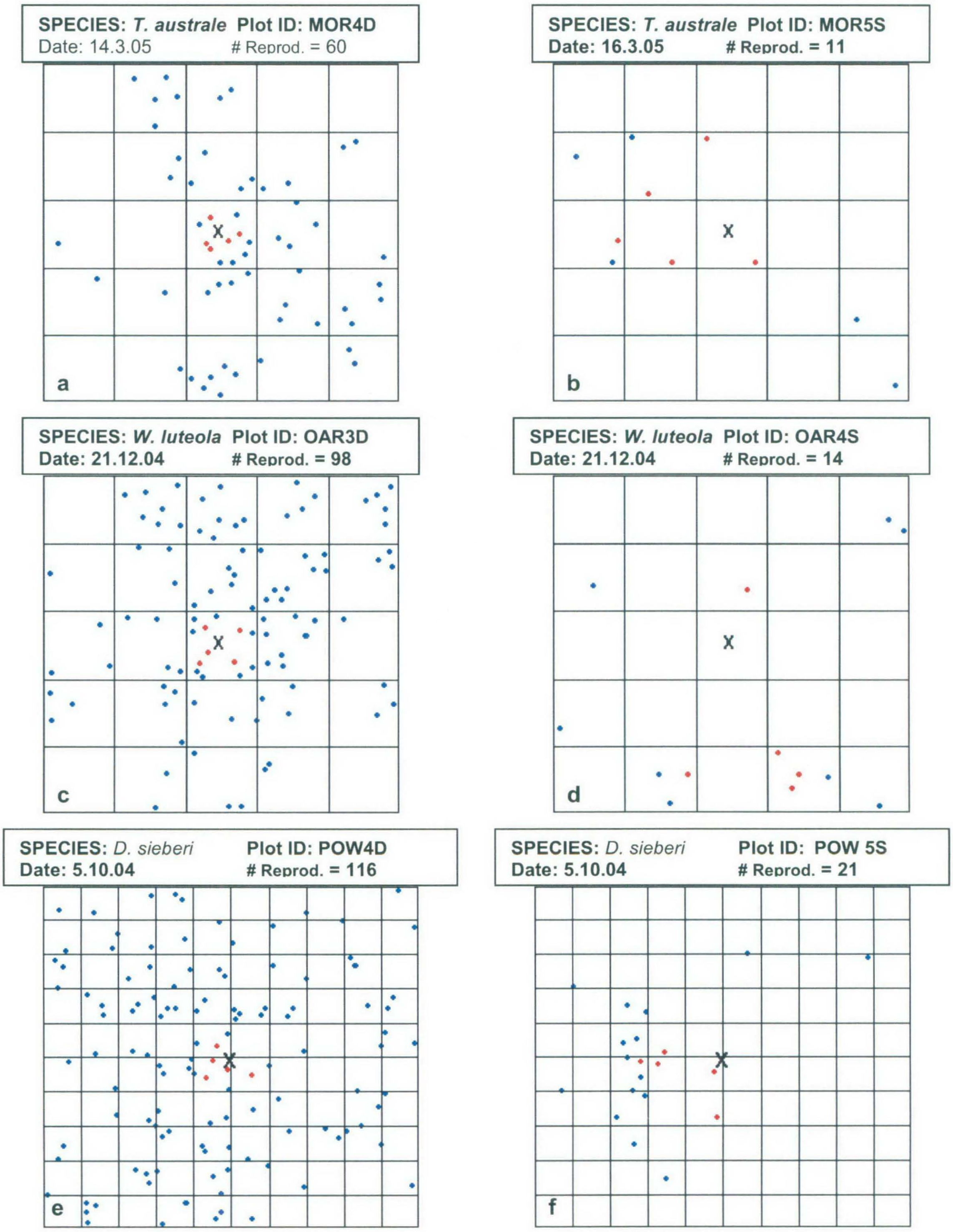

Figure 2.2 An example of plot maps for **a)** *T. australe* dense and **b)** *T. australe* sparse, **c)** *W. luteola* dense and **d)** *W. luteola* sparse and **e)** *D. sieberi* dense and **f)** *D. sieberi* sparse.
Reprod.=number of reproductive individuals within plot, X=focal plant, red dots=5 nearest reproductive neighbours, blue dots=reproductive individuals.

2.3.2 Habitat Attributes

To check whether there were differences in the local environment that may have been influential to relative density and the reproductive output of individuals, a number of habitat attributes were assessed within plots. To undertake this assessment, four of the $1m^2$ squares within each plot were randomly chosen (drawn out of a hat). A $1m^2$ quadrat was used to mark this area within the plot so it could be assessed for; height of tallest strata, and percent cover of grass, bare ground and leaf litter. The number and identity of other plant species occurring within plots were also recorded. The data from these $1m^2$ quadrats were then pooled for each plot for comparison within and among sites. Plant identification was assisted by referring to herbarium material, the Flora of New South Wales, local species lists and discussion with experts (C. Gross, I. Telford). A list of the species (for which there was adequate material for the purposes of identification) can be found in Appendix B.

2.4 Statistical Analyses

Analysis was undertaken using Statgraphics® *Plus* Version 5.1, 2001. One-way ANOVAs were used to analyse plot and habitat attributes. Data was checked for normality using Cochran's test. In addition, standard kurtosis and skewness values were checked to confirm normality. Non-normal data was transformed as necessary before analysis. Tukey's (HSD) was used to subsequently discriminate among means. If data could not be adequately transformed and normalised, a non-parametric test of the medians was undertaken; differences among medians were determined using box and whisker plots (median notch) as generated by the software package.

2.5 Results

2.5.1 Estimated population sizes, study plot densities and mapping

Estimated overall population sizes for each study species and site are presented in Table 2.1. For all species and all sites, populations were relatively large with individuals numbering in the hundreds or greater.

Table 2.1 Estimated population sizes for the three study species at each site.

SPECIES	SITE Estimated Number of Individuals		
T. australe	BM	ABR	MOR
	> 400	> 450	> 250
W. luteola	POW	OAR	UNE
	> 350	> 300	> 400
D. sieberi	POW	MOR	OAR
	> 1000	> 1000	> 500

Typical examples of sparse and dense plots for *T. australe*, *W. luteola* and *D. sieberi* are depicted in Figure 2.2 a-f. A full set of plot maps for each species and site can be found in Appendix A. To determine whether sparse and dense plots were distinctly different within sites and to see how they compared among sites, mean NND distances and the number of reproductive plants/m^2 (measures of absolute density) were analysed for each study species; results are presented in the following sections.

2.5.1.1 Thesium australe

Mean NND was significantly lower in dense plots than sparse for all sites (BM $F_{1,48}$ =43.74, P=0.000; ABR; $F_{1,48}$ =36.94, P=0.000 LOG transformed; MOR $F_{1,48}$ =114.03, P=0.000 LOG transformed). Mean NND among sites for dense plots was similar ($F_{2,72}$ =2.36, P=0.102), but ABR sparse plots had significantly lower mean NND's than BM or ABR ($F_{2,72}$ =5.77, P=0.005) (Figure 2.3). The mean number of reproductives/m^2 was significantly higher in dense plots than sparse for all sites (BM $F_{1,8}$ =24.23, P=0.001 LOG transformed; ABR $F_{1,8}$ =11.02, P=0.011 LOG transformed; MOR $F_{1,8}$ =36.50, P=0.000). The mean number of reproductives/m^2 was similar among sites for dense plots ($F_{2,12}$ =0.70, P=0.517), but ABR sparse plots contained significantly more reproductive individuals/m^2 than BM or MOR ($F_{2,12}$ =16.58, P=0.000). Therefore, although there were some minor among site differences for sparse plots, relative density differed for plots within all sites. Summary data for individual *T. australe* plots at each site are presented in Table 2.2.

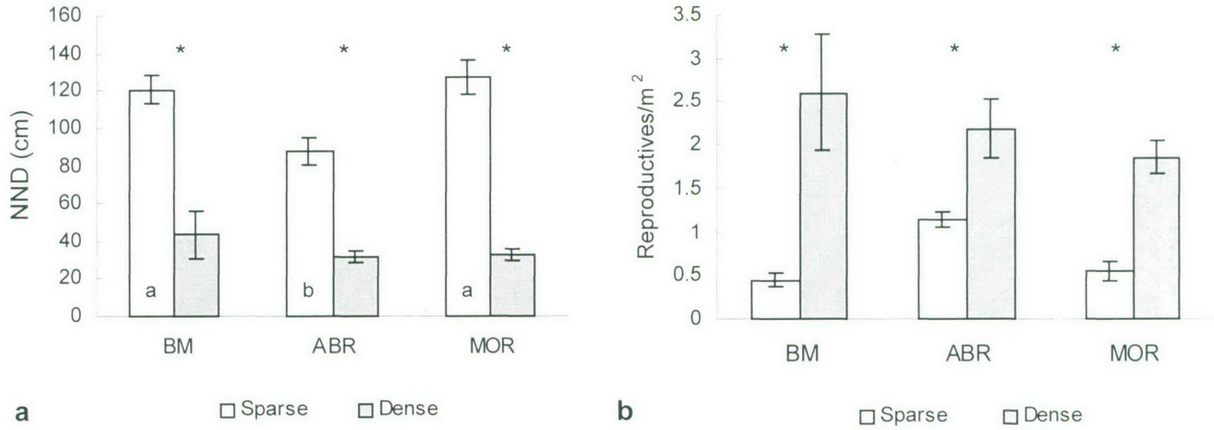

Figure 2.3 Measures of absolute density for *T. australe* plots at three sites **a)** Mean NND (±SE) (of the 5 nearest FP neighbours) and **b)** mean number (±SE) of reproductive plants/m^2. * = significant difference within site for density (P<0.05). Letters in base of columns indicate significant differences among site for density (P<0.05).

Table 2.2 Descriptive statistics of plot density attributes for three *Thesium australe* sites. BM=Black Mountain Cemetery, ABR=Aberfoyle Rd, MOR=Moray. S=sparse, D=dense.

PLOT ID	MEAN NND (cm)	Number of Reproductive Plants in Plot	Reproductive Plants/m^2 in Plot	Mean # Reproductive Plants in Plots ±SE	Range of Reproductives in Plots
BM1S	139.2	8	0.32	11.2±1.8	7-17
BM2S	127.4	17	0.68		
BM3S	112.2	11	0.44		
BM4S	130.0	7	0.28		
BM5S	94.4	13	0.52		
BM1D	50.4	54	2.16	65.0±16.7	20-122
BM2D	16.2	74	2.96		
BM3D	88.6	20	0.8		
BM4D	23.2	55	2.2		
BM5D	40.0	122	4.88		
ABR1S	74.4	29	1.16	28.8±2.29	23-36
ABR2S	95.8	23	0.92		
ABR3S	101.8	25	1.0		
ABR4S	108.8	36	1.44		
ABR5S	56.8	31	1.24		
ABR1D	15.6	79	3.16	54.8±8.53	34-79
ABR2D	27.6	34	1.36		
ABR3D	47.0	65	2.6		
ABR4D	38.2	59	2.36		
ABR5D	28.6	37	1.48		
MOR1S	180.8	41	0.48	14.0±2.77	35-60
MOR2S	95.0	42	0.48		
MOR3S	138.2	54	1.0		
MOR4S	100.6	60	0.4		
MOR5S	122.8	35	0.44		
MOR1D	28.0	12	1.64	46.4±4.59	10-25
MOR2D	49.6	12	1.68		
MOR3D	37.4	25	2.16		
MOR4D	27.8	10	2.4		
MOR5D	20.4	11	1.4		

2.5.1.2 Wahlenbergia luteola

Mean NND was significantly lower in dense plots than sparse within all sites (POW; $F_{1,48}$ =36.48, P=0.000; OAR $F_{1,48}$ =114.75, P=0.000 LOG transformed; UNE $F_{1,48}$ =46.17, P=0.000 LOG transformed). Mean NND's among sites for dense plots were similar ($F_{2,72}$ =0.36, P=0.699 LOG transformed), but UNE sparse plots had significantly smaller mean NND's than OAR ($F_{2,72}$ =0.36, P=0.031) (Figure 2.4). The mean number of reproductives/m^2 was similar among sites for dense plots ($F_{2,12}$ =0.11, P=0.901), but OAR plots contained significantly fewer reproductive individuals/m^2 than UNE ($F_{2,12}$ =6.70, P=0.011). Therefore, although there were some minor differences among sites for sparse plots, there was an overall difference in relative density between plot types across all sites. Summary data for individual *W. luteola* plots at each site are presented in Table 2.3.

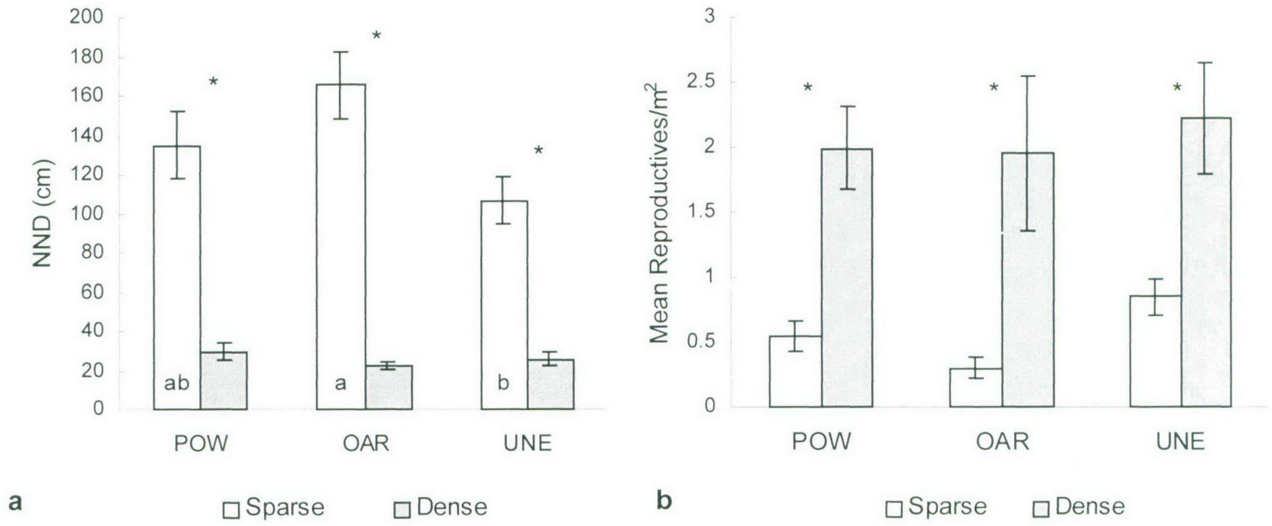

Figure 2.3 Measures of absolute density for *W. luteola* plots at three sites **a)** Mean NND (±SE) (of the 5 nearest FP neighbours) and **b)** mean number (±SE) of reproductive plants/m^2. *=Significant difference within site for density (P<0.05). Letters in base of columns indicate significant differences among site for density (P<0.05).

Table 2.3 Descriptive statistics of plot density attributes for three *Wahlenbergia luteola* sites POW=Powalgarh, ABR=Aberfoyle Rd, UNE=University of New England. S=sparse, D=dense.

PLOT ID	MEAN NND (cm)	Number of Reproductive Plants in Plot	Reproductive Plants/m^2 in Plot	Mean # Reproductive Plants in Plots ±SE	Range of Reproductives in Plots
POW1S	112.8	25	1.0	13.4±2.98	8-25
POW2S	123.8	12	0.48		
POW3S	140.6	8	0.32		
POW4S	147.8	11	0.44		
POW5S	149.4	11	0.44		
POW1D	46.0	36	1.44	49±8.1	36-78
POW2D	18.8	78	3.12		
POW3D	41.4	40	1.6		
POW4D	13.4	58	2.32		
POW5D	27.4	37	1.48		
OAR1S	133.6	9	0.36	7.4±2.09	2-14
OAR2S	223.6	2	0.08		
OAR3S	195.2	4	0.15		
OAR4S	176.4	14	0.56		
OAR5S	99.8	8	0.32		
OAR1D	17.0	30	1.2	48.8±14.71	20-98
OAR2D	23.2	29	1.16		
OAR3D	27.8	98	3.92		
OAR4D	20.4	67	2.68		
OAR5D	23.8	20	0.8		
UNE1S	93.4	15	0.6	20.8±3.60	14-34
UNE2S	78.4	22	0.88		
UNE3S	45.4	34	1.36		
UNE4S	178.2	19	0.76		
UNE5S	138.0	16	0.56		
UNE1D	13.0	37	1.48	55.4±10.73	37-96
UNE2D	32.4	96	3.84		
UNE3D	42.0	54	2.16		
UNE4D	20.2	38	1.52		
UNE5D	21.6	52	2.08		

2.5.1.3 Dillwynia sieberi

Mean NND was significantly lower in dense plots than sparse within all sites (POW H =26.75, P<<0.000; OAR $F_{1,48}$ =25.78, P=0.000 LOG transformed; UNE H = 28.49, P<<0.000 Kruskal Wallis comparison of medians). Mean NND among sites for sparse plots were similar for POW and MOR, but were significantly higher at OAR ($F_{2,72}$ =10.35, P=0.000 LOG transformed) and NNDs for dense plots were higher at OAR than for POW ($F_{2,72}$ =3.34, P=0.041) (Figure 2.5). The mean number of reproductives/m^2 for sparse plots was similar at POW and MOR but lower at OAR ($F_{2,12}$ =9.14, P=0.004 LOG transformed), but OAR plots contained significantly fewer reproductive individuals/m^2 than UNE ($F_{2,12}$ =6.70, P=0.011). All sites varied for number of reproductives/m^2 (POW>MOR>OAR $F_{2,12}$ = 43.03, P=0.000 LOG transformed). Therefore, there were some differences among sites for sparse and dense plots, however there were clear relative density differences between plot types across all sites. Summary data for individual *D. sieberi* plots at each site are presented in Table 2.4.

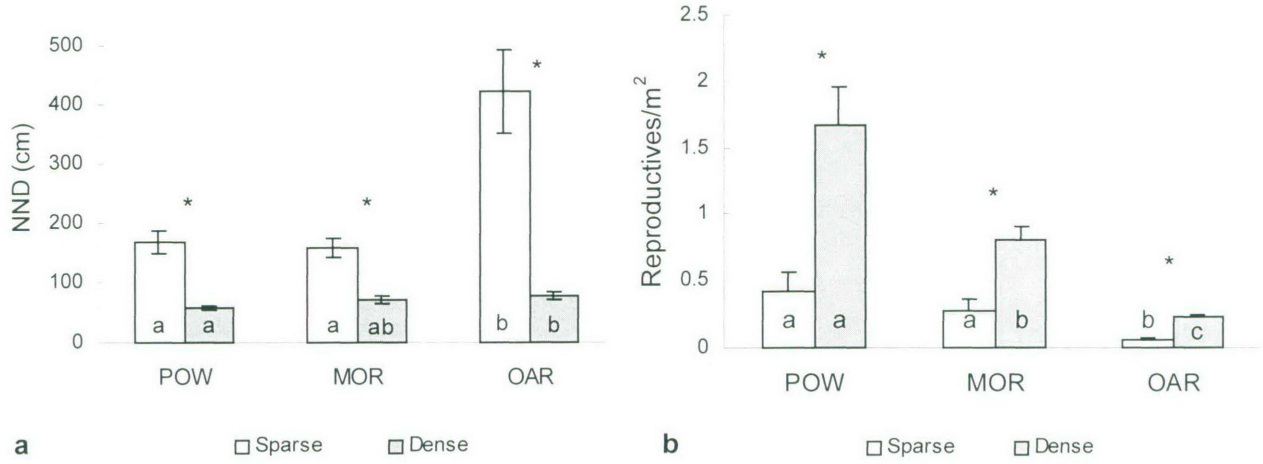

Figure 2.5 Measures of absolute density for *D. sieberi* plots at three sites **a)** Mean NND (±SE) (of the 5 nearest FP neighbours) and **b)** mean number (±SE) of reproductive plants/m^2. *=Significant difference within site for density (P<0.05). Letters in base of columns indicate significant differences among site for density (P<0.05).

Table 2.4 Descriptive statistics of plot density attributes for three *Dillwynia sieberi* sites POW=Powalgarh, MOR=Moray, OAR=Old Armidale Rd. S=sparse, D=dense.

PLOT ID	MEAN NND (cm)	Number of Reproductive Plants in Plot	Reproductive Plants/m^2 in Plot	Mean # Reproductive Plants in Plots ±SE	Range of Reproductives in Plots
POW1S	89.6	91	0.91	42.0±13.57	13-91
POW2S	250	13	0.13		
POW3S	168	42	0.42		
POW4S	155.4	43	0.43		
POW5S	168.6	21	0.21		
POW1D	54.2	222	2.22	166.2±28.59	81-222
POW2D	63.6	218	2.18		
POW3D	42.6	194	1.94		
POW4D	62.8	116	1.16		
POW5D	62.6	81	0.81		
MOR1S	135	20	0.20	26.8±9.10	7-61
MOR2S	227.6	20	0.20		
MOR3S	83.4	61	0.61		
MOR4S	193.2	26	0.26		
MOR5S	145.8	7	0.07		
MOR1D	47.2	99	0.99	81.2±9.08	54-99
MOR2D	74.8	95	0.95		
MOR3D	75	93	0.93		
MOR4D	58.8	65	0.65		
MOR5D	100.2	54	0.54		
OAR1S	128.4	10	0.10	6.0±1.67	2-10
OAR2S	504	4	0.04		
OAR3S	282.2	10	0.10		
OAR4S	514.4	4	0.04		
OAR5S	676	2	0.02		
OAR1D	62.2	17	0.17	22.4±2.6	16-29
OAR2D	51	29	0.29		
OAR3D	71.6	27	0.27		
OAR4D	99.4	23	0.23		
OAR5D	100.4	16	0.16		

2.5.2 Habitat Attributes

The habitat attribute data collected from the plots within each of the six sites were initially pooled (for site) to gain a general impression of how sites compared for each attribute. The data were then compared for density on a species-by-site basis. In general, plant density was not obviously related to any of the habitat attributes measured for any of the study species. With a few exceptions, most variables were relatively homogenous across individual study sites, and significant differences occurred mainly among sites rather than within (between densities). A summary of analysis results is presented in Appendix C.

2.5.2.1 Overview of study sites

Species richness was highest at Aberfoyle Road followed by OAR followed by all other sites (H=106.149, P=0.000, Kruskal-Wallis comparison of the medians). Height of the tallest stratum was similar across POW, MOR and ABR, but varied among the remaining three sites (H=90.61, P=0.000, Kruskal-Wallis comparison of the medians). There was no difference among sites in the percentage of bare ground ($F_{1,121}$=1.98, P=0.087 1/sqrt transformed). Grass cover was reasonably high but variable for all sites, except at UNE where it was less than 50% (H=87.03, P=0.000). There was statistically significant among-site variability for most of the attributes measured, but overall none appear to be a dominant attribute.

2.5.2.2 Thesium australe

Density of *T. australe* individuals was associated with increased species richness, a higher incidence of bare ground and lower incidence of grass cover. However, this was neither consistent nor significant across all sites. Species richness was significantly higher in dense than sparse plots at ABR ($F_{1,38}$ =17.98, P=0.000) and MOR ($F_{1,38}$ =9.92, P=0.003) but not BM ($F_{1,38}$ =1.83, P=0.184). At all sites, percent grass cover was lower in dense plots but this was significant only at MOR ($F_{1,38}$ =4.94, P=0.032). No patterns could be discerned with regard to the other variables measured (Figure 2.7).

2.5.2.3 Wahlenbergia luteola

There were few indications that *W. luteola* density was influenced by the habitat variables measured. The number of species present within sparse and dense plots did not differ with the exception of OAR, where dense plots contained a greater number of species (POW $F_{1,38}$ =0.10, P=0.753; OAR $F_{1,38}$ =11.21, P=0.002; UNE $F_{1,34}$ =0.15, P=0.703). Dense plots were found in areas of higher leaf litter cover at OAR ($F_{1,38}$ =51.73, P=0.000) and higher grass cover at UNE ($F_{1,33}$ =7.94, P=0.008) but these variable were similar between densities at the remaining sites. Again, there were no clear trends revealed in terms of density and the variables measured (Figure 2.8).

2.5.2.4 Dillwynia sieberi

Percent leaf litter cover and grass cover appeared to be correlated with *D. sieberi* density at POW and MOR, but not at OAR. Dense plots were found in areas of higher leaf litter (POW $F_{1,38}$ =6.69, P=0.014; MOR $F_{1,38}$ =11.41, P=0.002) and in areas of reduced grass cover (POW $F_{1,38}$ =7.84, P=0.008; MOR H =4.97, P=0.026 Kruskal-Wallis comparison of medians). There were no other obvious trends (Figure 2.9).

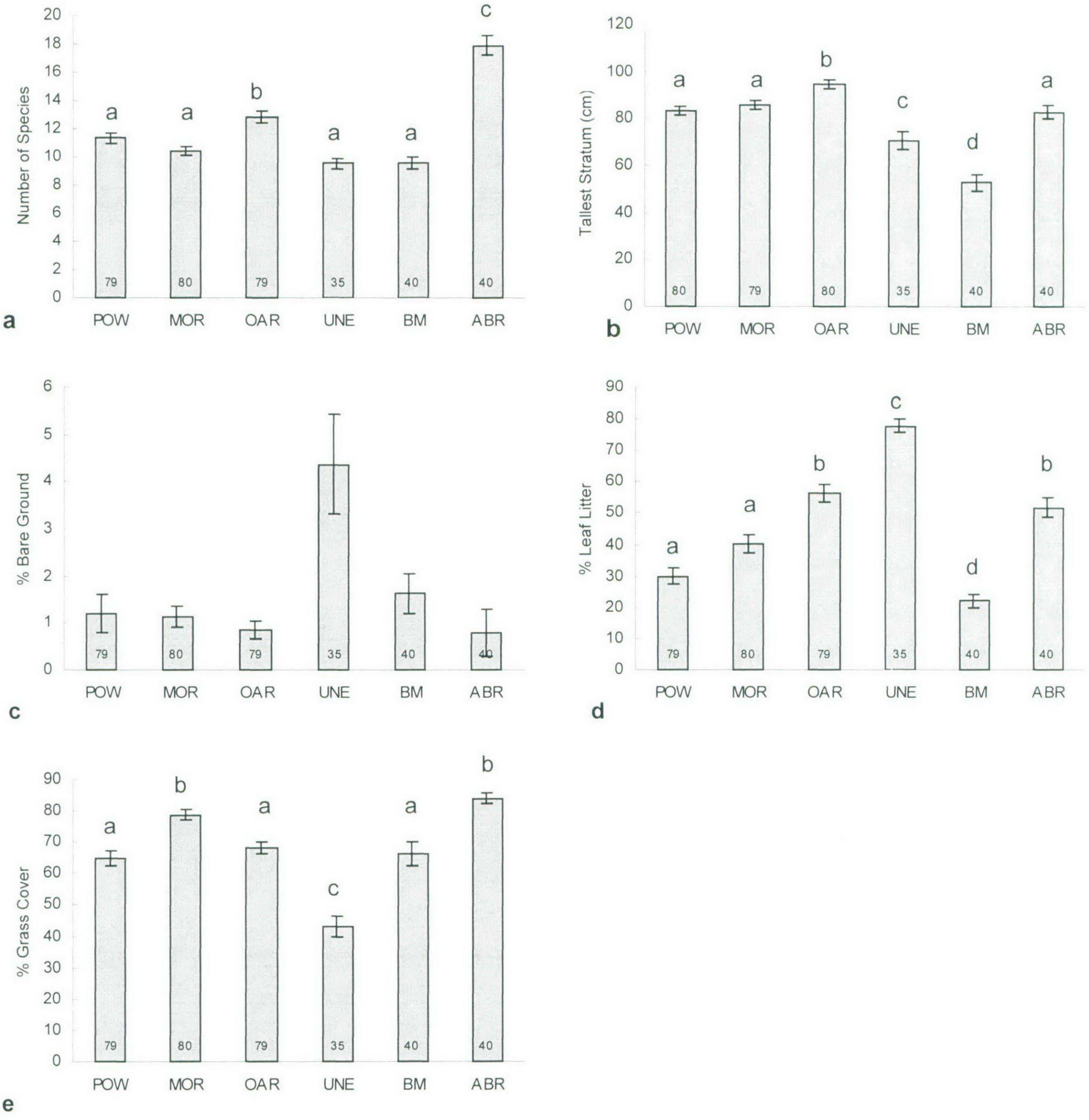

Figure 2.6 Habitat attribute assessment of the six study sites **a)** Number of species **b)** Height of the tallest strata **c)** % bare ground **d)** % leaf litter cover **e)** % grass cover. Means are ±SE. N (in base of columns)=number of $1m^2$ quadrats. Letters above columns denote significant differences among sites (P<0.05). See table 1.1 for an explanation of site abbreviations.

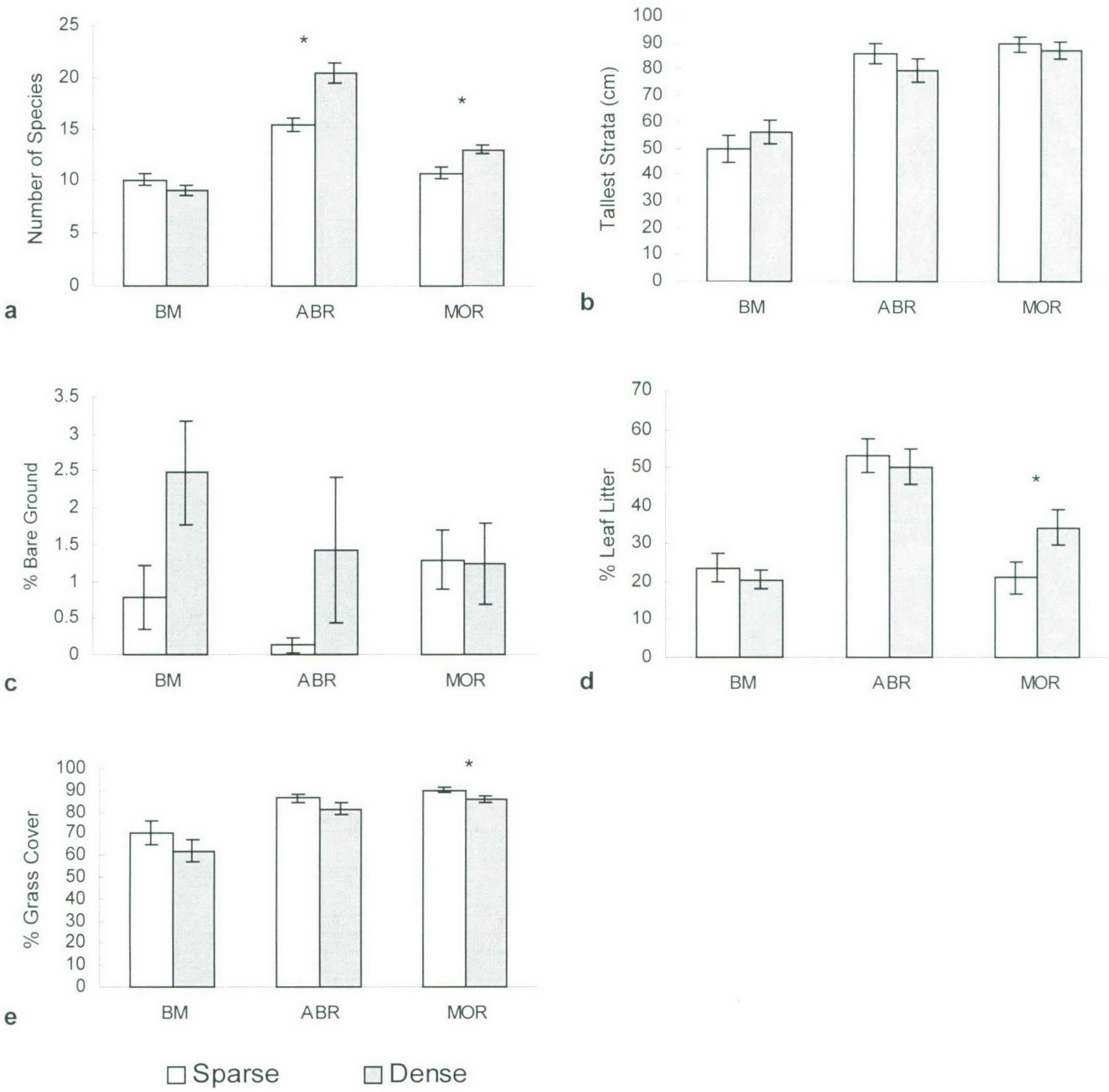

Figure 2.7 Habitat attribute assessment of *T. australe* sparse and dense plots at three sites **a)** Number of species **b)** Height of the tallest strata **c)** % bare ground **d)** % leaf litter cover **e)** % grass cover. Means are ±SE. * Above columns denotes significant differences within site for density (P<0.05). See table 1.1 for an explanation of site abbreviations.

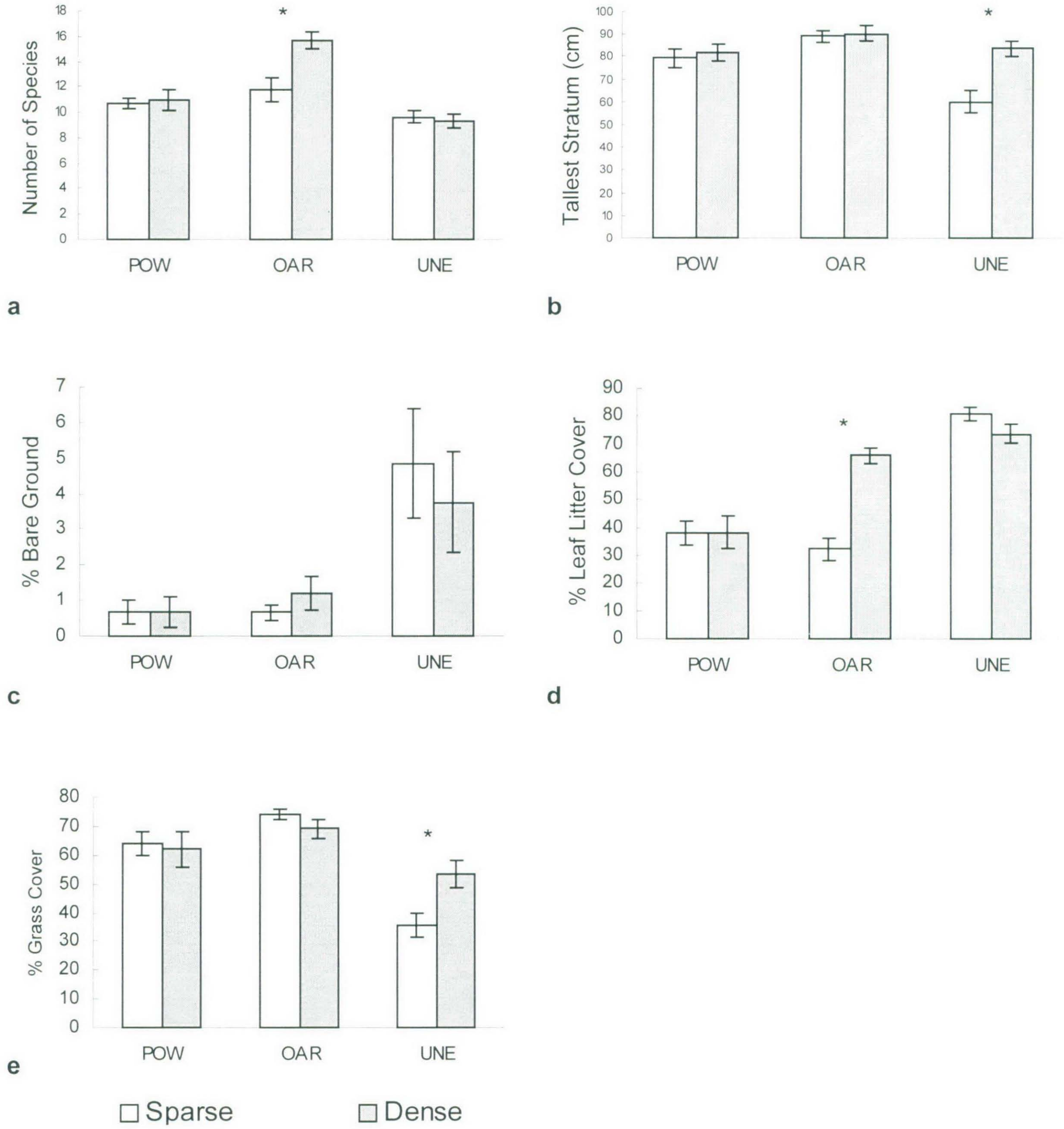

Figure 2.8 Habitat attribute assessment of *W. luteola* sparse and dense plots at three sites **a)** Number of species **b)** Height of the tallest strata **c)** % bare ground **d)** % leaf litter cover **e)** % grass cover. Means are ±SE. * Above columns denotes significant differences within site for density (P<0.05). See table 1.1 for an explanation of site abbreviations.

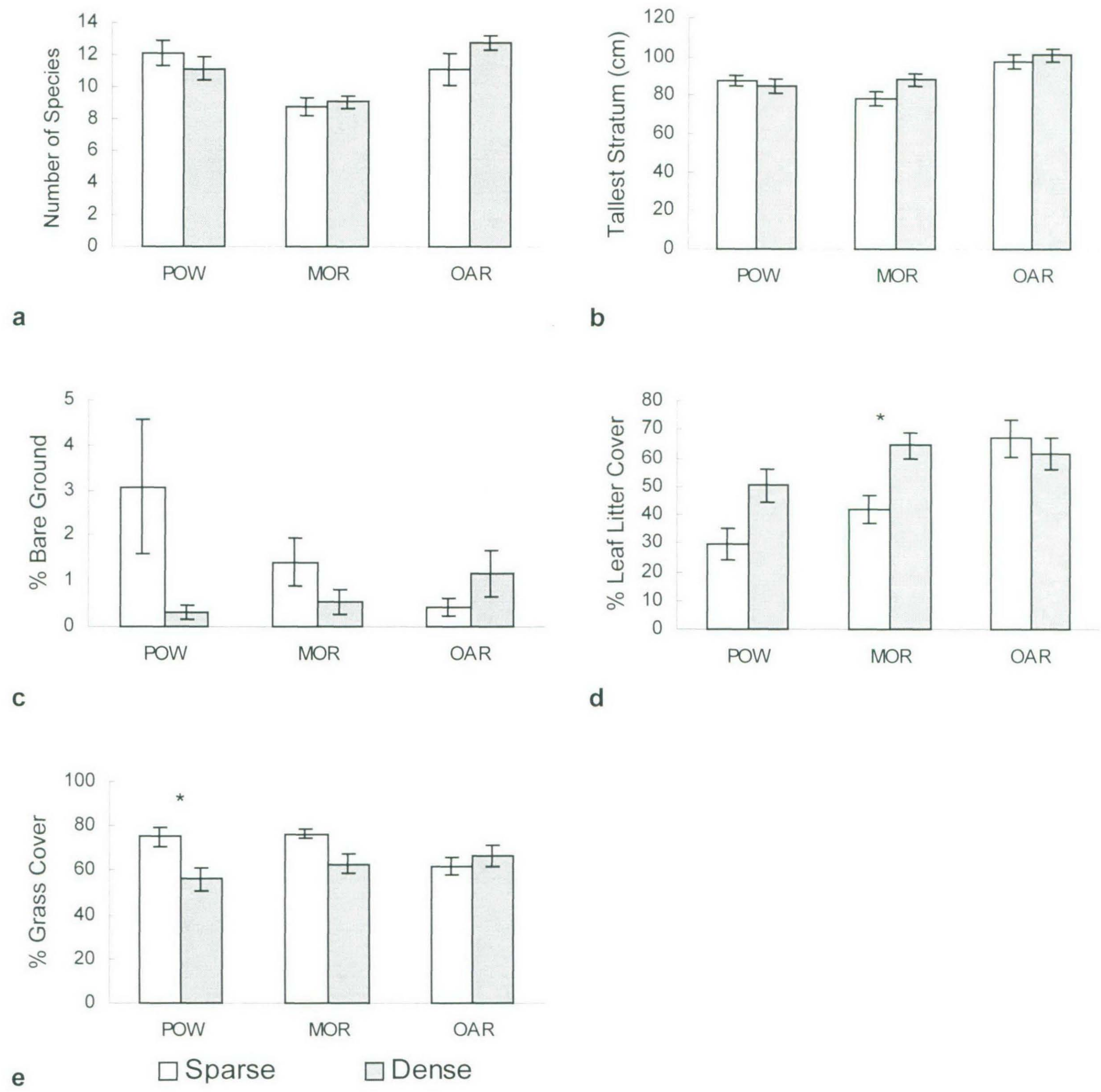

Figure 2.9 Habitat attribute assessment of *D. sieberi* sparse and dense plots at three sites **a)** Number of species **b)** height of the tallest strata **c)** % bare ground **d)** % leaf litter cover **e)** % grass cover. Means are ±SE. * Above columns denotes significant differences within site for density (P<0.05). See table 1.1 for an explanation of site abbreviations.

2.6 DISCUSSION

The results clearly establish that measurements of both absolute and relative density of reproductive individuals were significantly different between densities within sites and for all study species. There was however, some variability in absolute density measures (both at the plot level and at the FP level) among sites for each species. This is to be expected as the study was undertaken in a natural system. Although considerable effort was afforded in an attempt to establish plots that were as similar in their relevant densities as possible, plots ultimately reflected the range of densities that occur naturally in these systems and no manipulations (such as plant or flower removal) were undertaken to standardise them.

There is some evidence to suggest that *T. australe* recruitment can be negatively impacted upon via competition from shrubs and grasses (including one of its host plants *Themeda australis*) (Cohn 2004). Thus, the tendency for *T. australe* density to be higher in areas that provision relatively more bare ground and less grass cover may indicate that competition is affecting recruitment within the sparser *T. australe* patches. However, demographic processes would need to be investigated to determine this. Apart from this, there were few indications that plant density was influenced by any of the habitat attributes measured for any of the plant species and that site differences were negligible.

In the chapters that follow, the density parameters established here will be utilised to determine what influence if any, they impose on aspects of visitation, reproductive output and fitness for each of the study species. The breeding systems of the study species however, must first be established.