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Introduction

The microbiota present in pruning wounds of apricot wood differed when the trees were sheltered from rainfall. Some microbes may compete with the causal fungus of *Eutypa dieback*. Similar experiments have not been done on grapevines. The presence of microbes observed over time in wound surfaces of grapevine canes following pruning under shelter or exposed to rainfall was examined.

Methods

Vines were spur-pruned and were exposed to rainfall or under shelters (Fig. 1). Shelters excluded all rainfall from season break until bud-burst (Fig. 2a). In 2016 and again in 2017 on each of five sampling days over the 28 days post-pruning, a single node was removed from a randomly selected spur from each vine. Nodes were flame sterilised, and both green and necrotic wood from the cut surface were sampled by making lengthwise and transverse cuts (Fig. 2b). Fragments were applied to media (nutrient agar in 2016 only and potato dextrose agar (PDA) plus streptomycin in both years) (Fig. 2c). Fungal cultures that grew were identified according to morphology. The most frequently observed fungi were identified by nucleotide sequencing. In 2017 the predominantly occurring fungi in wounds exposed to rainfall were co-cultured with *Eutypa lata*.

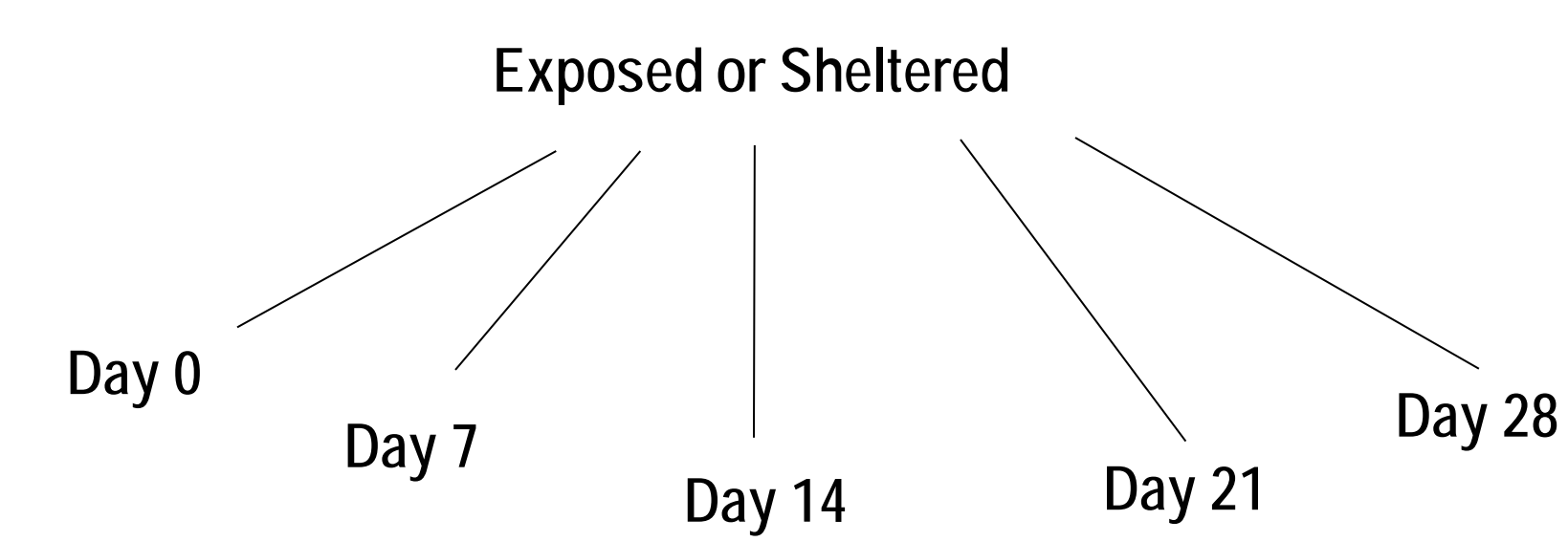


Figure 1. Experimental plan.

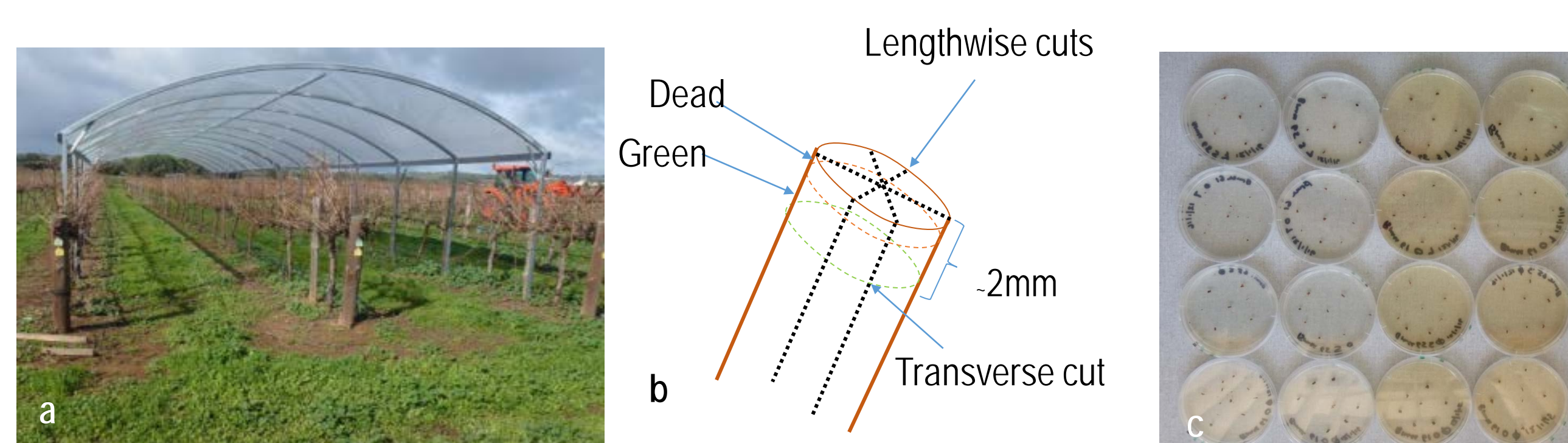


Figure 2. Rainfall exclusion (a), cane processing (b), plates prepared for incubation (c).

Results

By day 21 in 2016 a clear trend had emerged on exposed (Fig. 3a) and sheltered vines (Fig. 3b). Bacteria were rarely recovered. The species of fungi present appeared to differ between treatments (Fig. 4). The most frequently occurring species were identified as *Alternaria* spp. (Fig. 5a), *Aureobasidium pullulans* (Fig. 5b), *Cladosporium* spp. (Fig. 5c), and *Epicoccum nigrum* (Fig. 5d).

E. nigrum appeared more frequently in rainfall exposed than in sheltered wounds.

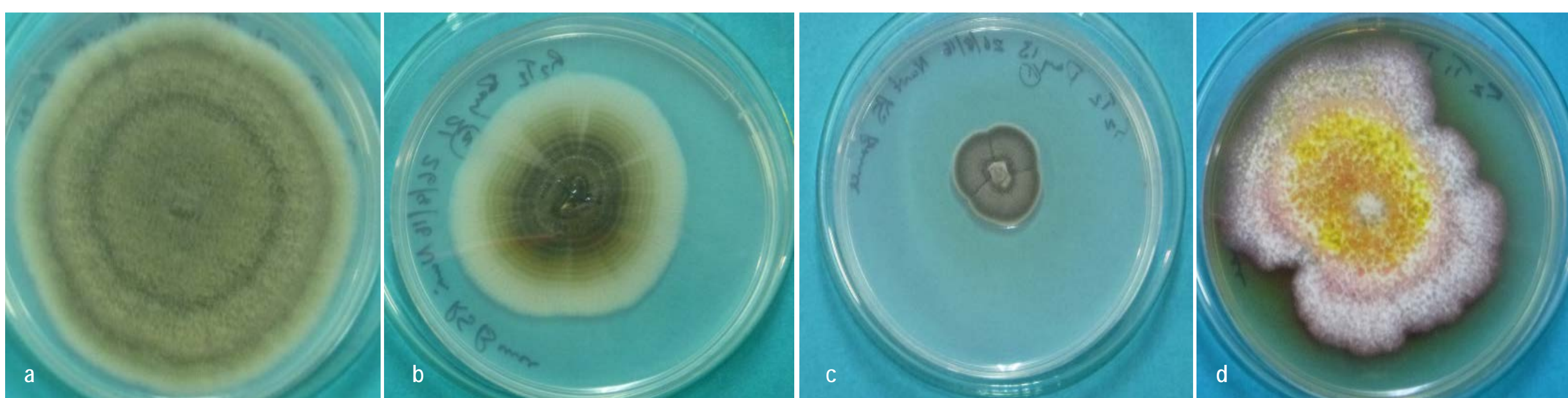


Figure 5. Most frequently observed fungi in 2016. *Alternaria* spp. (a), *Aureobasidium pullulans* (b), *Cladosporium* spp. (c), *Epicoccum nigrum* (d).

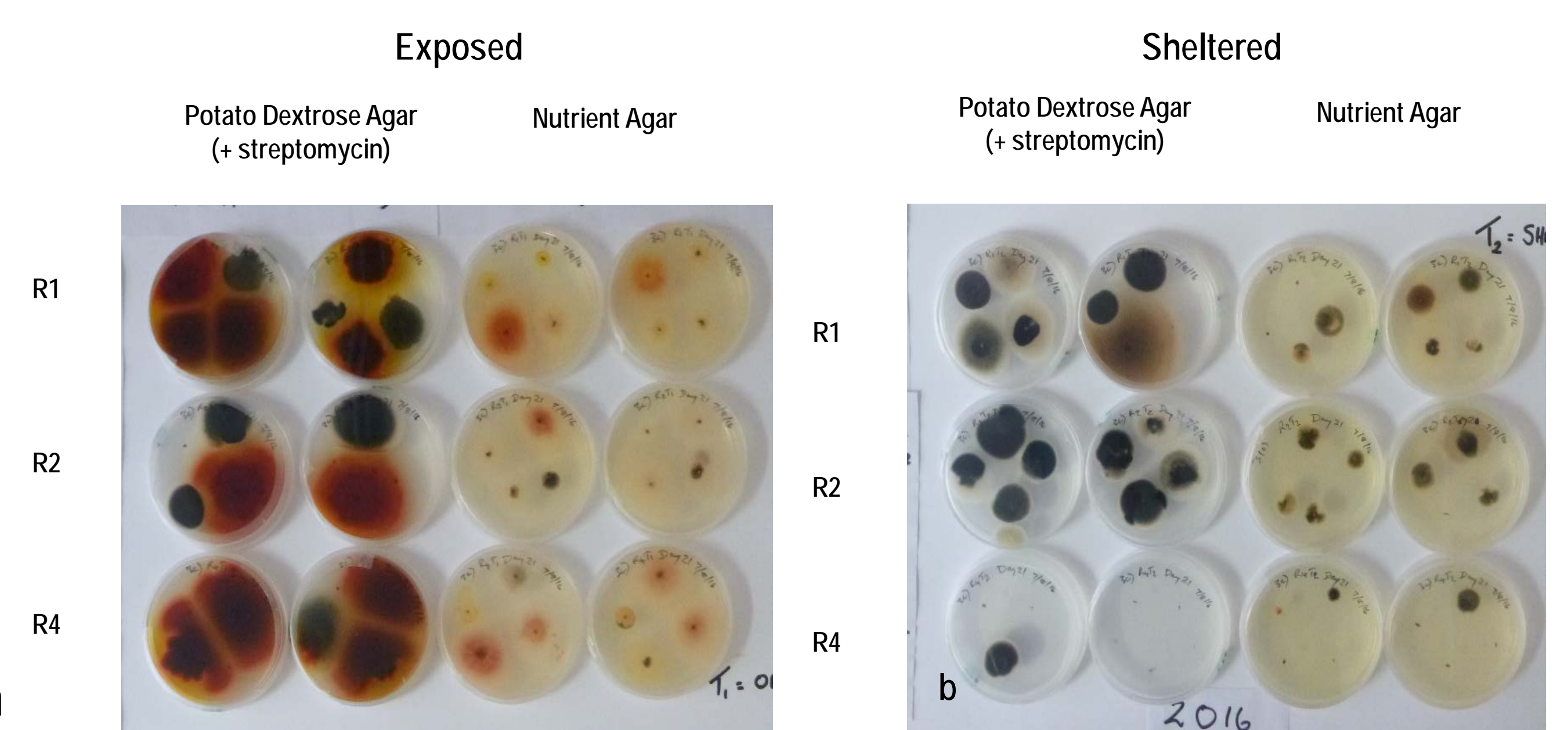


Figure 3. Fungi isolated, 2016 sampling day 21: rainfall exposed (a) and sheltered (b).

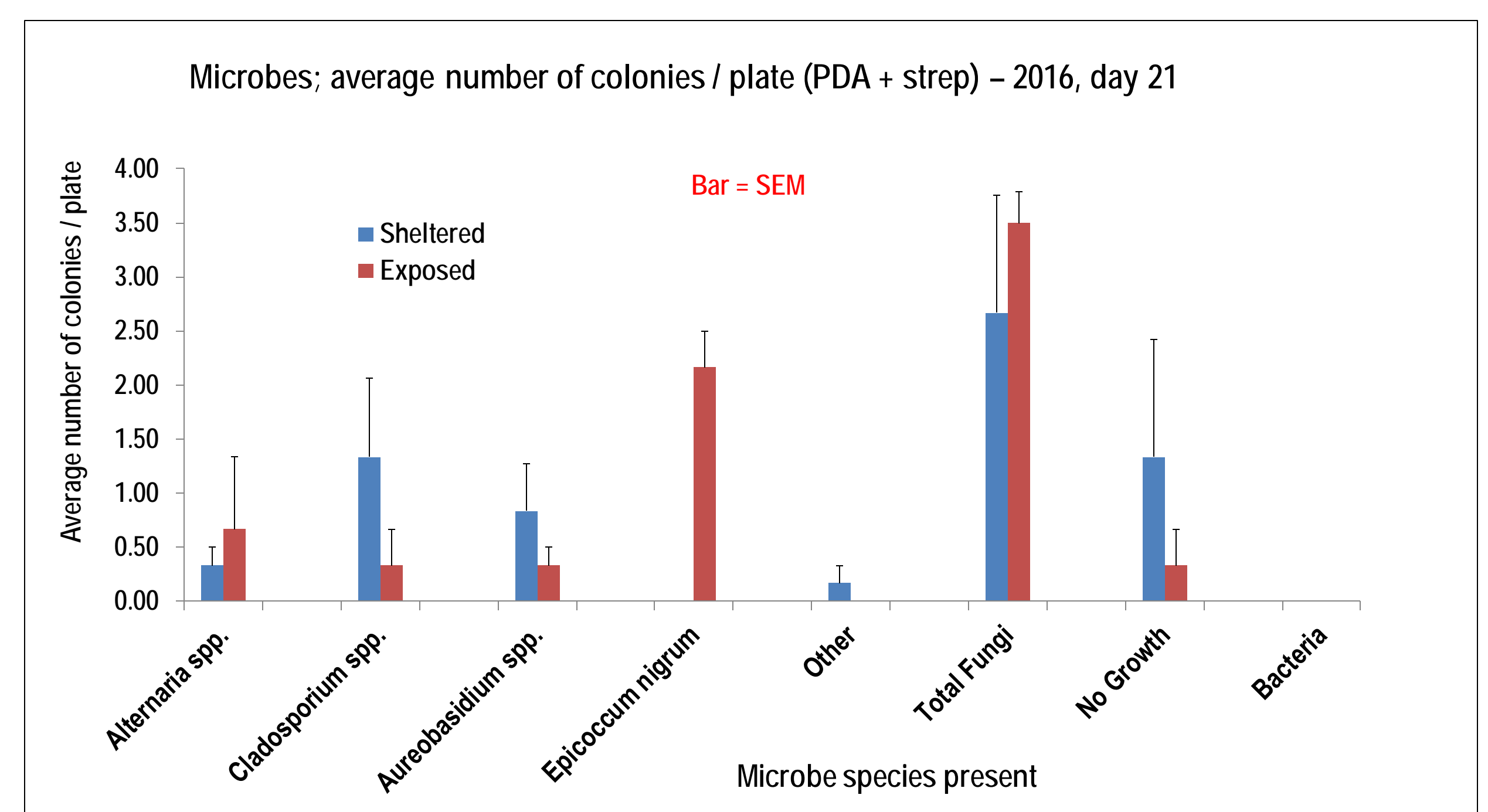


Figure 4. Average numbers of microbe species present - 2016, sampling day 21.

In 2017, two frequently occurring cultures of similar appearance were isolated (Fig. 6), predominantly from rainfall exposed wounds. They were identified as *Epicoccum* sp. and *E. nigrum*. Their sequences differed by one base pair.

The results of co-culturing these fungi with *E. lata* suggests that *E. nigrum* competes for space with, rather than being antagonistic to, *E. lata* (Fig. 7a,b).

Conclusion

E. nigrum was the dominant fungal species identified in the pruning wounds of grapevines exposed to rainfall. *E. nigrum* has been used in the control of other fungal pathogens. Further investigation will be required to determine whether *E. nigrum* has an influence on the ecology of *E. lata* or other fungal pathogens that cause grapevine trunk disease.

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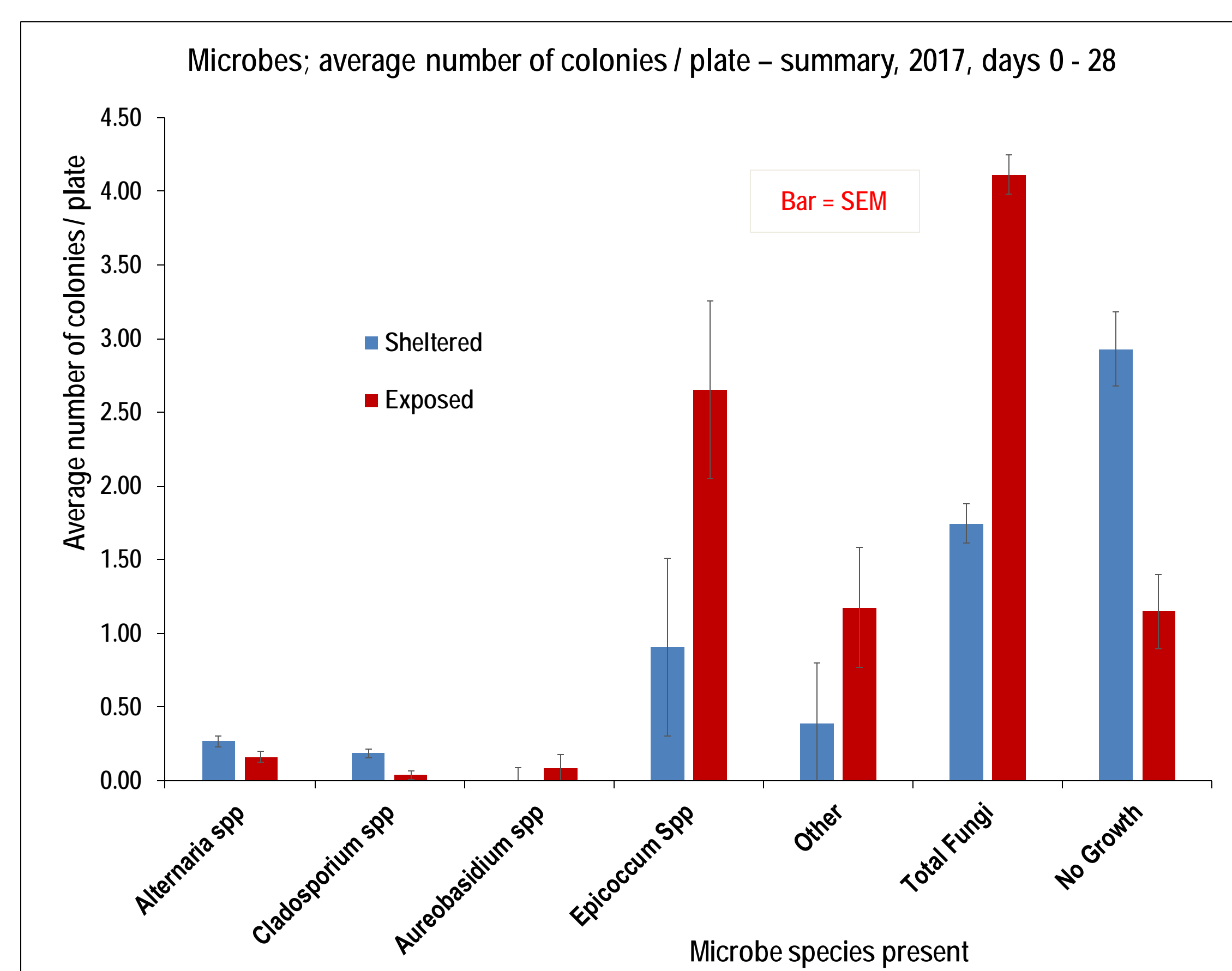


Figure 6. Average numbers of microbe species present - summary 2017, sampling days 0-28.

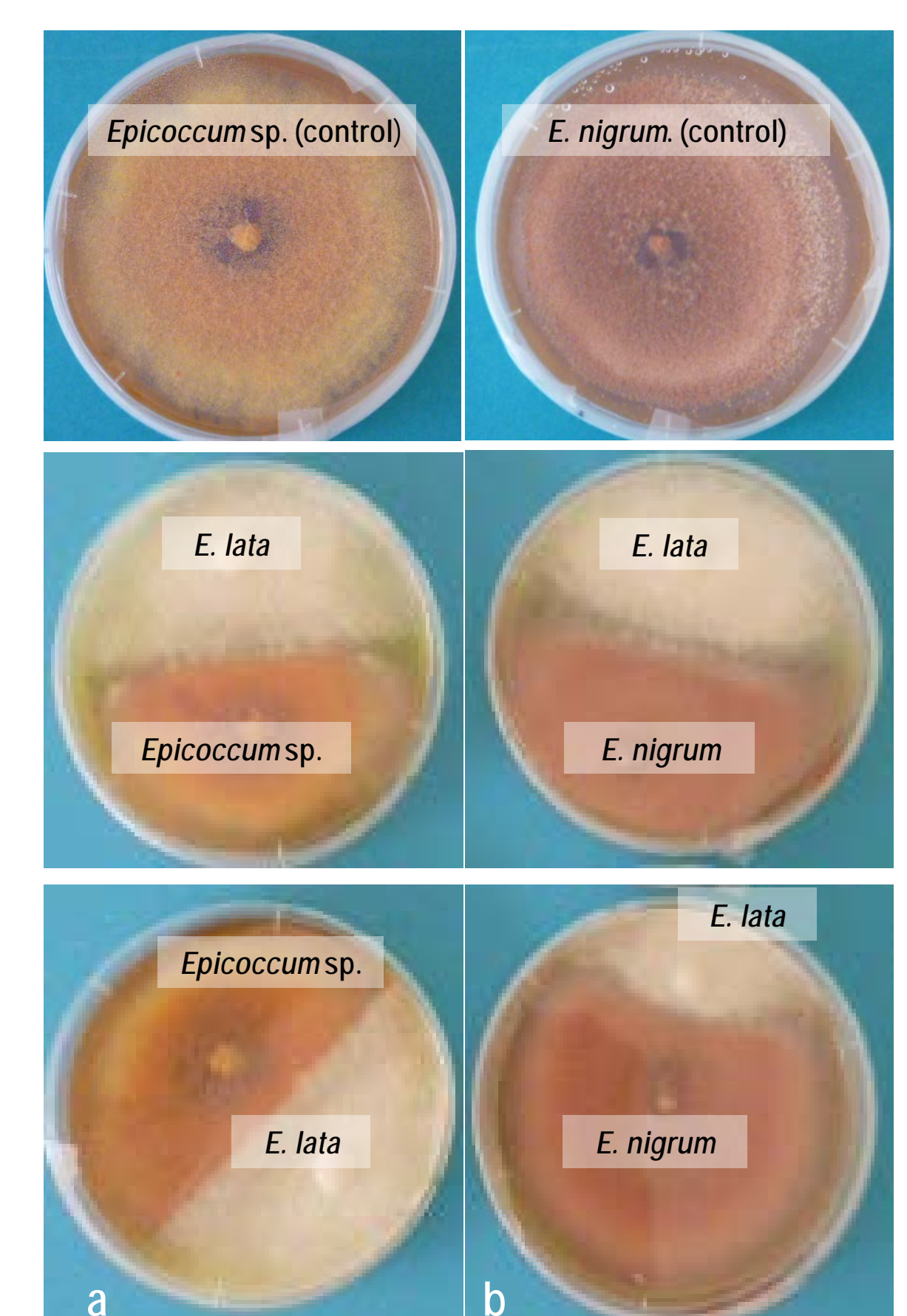


Figure 7. Dual cultures of *Epicoccum* sp. with *Eutypa lata* (a), and *E. nigrum* with *E. lata* (b).