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# Appendix C from T. Bell et al., "A Linear Model Method for Biodiversity–Ecosystem Functioning Experiments"

## (Am. Nat., vol. 174, no. 6, p. 836)

## Methodology for the Experiments Described in "Application"

### Bacterial Experiment

The experimental methods are described by Bell et al. (2005). Briefly, a pool of 72 bacterial isolates was obtained from an aquatic ecosystem. These were inoculated into microcosms containing sterile leaf litter and water. Communities were assembled according to the random partition design described in the text, with $M = 687$, $Q = 5$, and $n = 2$ and where all factors of 72 species were used (1, 2, 3, 4, 6, 8, 12, 18, 24, 36, 72). Total community respiration ($\mu$g $CO_2$ $mL^{-1}$ $day^{-1}$) was used as the measure of ecosystem functioning, and the experiment lasted for 28 days. The data that are analyzed reflect the total amount of $CO_2$ evolved over the course of the experiment.

### Plant Experiment

The random partitions experimental design (Bell et al. 2005) was used to construct 216 grass communities with species richness levels of 1, 2, 3, or 6 species. At each richness level, species were chosen randomly from the species pool without replacement. The grass species used in this study were redtop (*Agrostis gigantea*), creeping bentgrass (*Agrostis stolonifera*), tall fescue (*Lolium arundinaceum*), perennial ryegrass (*Lolium perenne*), Canada bluegrass (*Poa compressa*), and Kentucky bluegrass (*Poa pratensis*). We used nine 0.82 × 0.45 × 0.52-m (L × W × H) Plexiglas chambers arranged in a 3 × 3 square in the greenhouse. The chambers were controlled by an Argus greenhouse control system that continuously adjusted $CO_2$ concentrations, relative humidity, and temperature. For more information on the chamber construction and operation, see article by Grodzinski et al. (1999). In addition to the diversity manipulation, we also used three levels of $CO_2$: ambient, 500 ppm, and 600 ppm. The $CO_2$ treatment had no significant effect on total dry mass and so, for the purposes of this study, has been ignored.

Using the results from a germination study, we standardized the timing of seed planting with the goal of having each species germinate simultaneously. In addition, the germination success rate for each species was used when determining the number of seeds of each species to plant. In all cases we aimed to achieve an initial target community of 60 plants per pot. This target was chosen to achieve equal numbers of each species in any given mixture (e.g., 10 plants/species in the six-species mixture). We used 8.89 × 38.1-cm (diameter × height) circular pots made from 0.635-cm-thick PVC piping. The pots were filled with Sunshine Mix 1 potting medium and watered to field capacity daily until the start of the experiment and two to three times per week thereafter as needed. Each chamber contained 24 pots and two complete random partitions of the six-species pool. One partition comprised six monoculture pots, three two-species pots, two three-species pots, and one six-species pot.

Pots (communities) were placed randomly within each growth chamber and grown for 14 weeks (beginning September 4, 2007). Each pot was watered regularly with deionized water and clipped as needed to maintain approximately 6 cm canopy surface height. There were seven such "trimmings" in all. The clippings were recovered and dried, and their dry mass was included in the calculations of final dry matter production. After 14 weeks, a complete harvest of all plant material above- and belowground was conducted. Roots and shoots were separated, dried, and weighed. The summed dry mass from each harvest and final root and shoots was used here as our measure of ecosystem functioning.

### Literature Cited Only in Appendix C

Grodzinski, B., J. M. Schmidt, B. Watts, J. Taylor, S. Bates, M. A. Dixon, and H. Staines. 1999. Regulating plant/insect interactions using $CO_2$ enrichment in model ecosystems. Advances in Space Research 24:281–291.