

Understanding Differences in the Biology of Domestic and Imported Cultivars of Hops (*Humulus lupulus* L.) in San Diego

CLASS OF BIOL 596 RESEARCH METHODS IN AGRICULTURAL SCIENCES (FALL 2023)

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INTRODUCTION

- Humulus lupulus* L., or domestic hops are widely cultivated in the United States, primarily to supply the brewing industry.
- Prized for their differential concentrations of alpha (humulones) and beta (lupulones) acids, there are over 100 commercially available hop cultivars derived from five varietals of *H. lupulus*.
- San Diego county, once a thriving site for hop farming has since fallen behind, with extreme drought, and spread of fungal, bacterial, and viral pathogens.
- Previous studies have shown significant differences in growth rates between domesticated cultivars from across the world (Acosta-Rangel et al., 2021); while also developing new genomic resources for commercial hop production (e.g. alpha and beta acid concentrations – Padgett-Cobb et al., 2023).
- Understanding hop biology in a common-garden setting is therefore key to re-invigorating this lucrative industry.

QUESTIONS

- What are the differences in soil microbial and fungal communities between US and non-US based cultivars?
- What are the functional consequences of differences in microbial and fungal species diversity in growth rates?
- How do differences in microbial species diversity correlate with soil nitrogen, phosphorous, and moisture levels?
- How can we utilize Next Generation Sequencing, and metagenomics to address these questions?

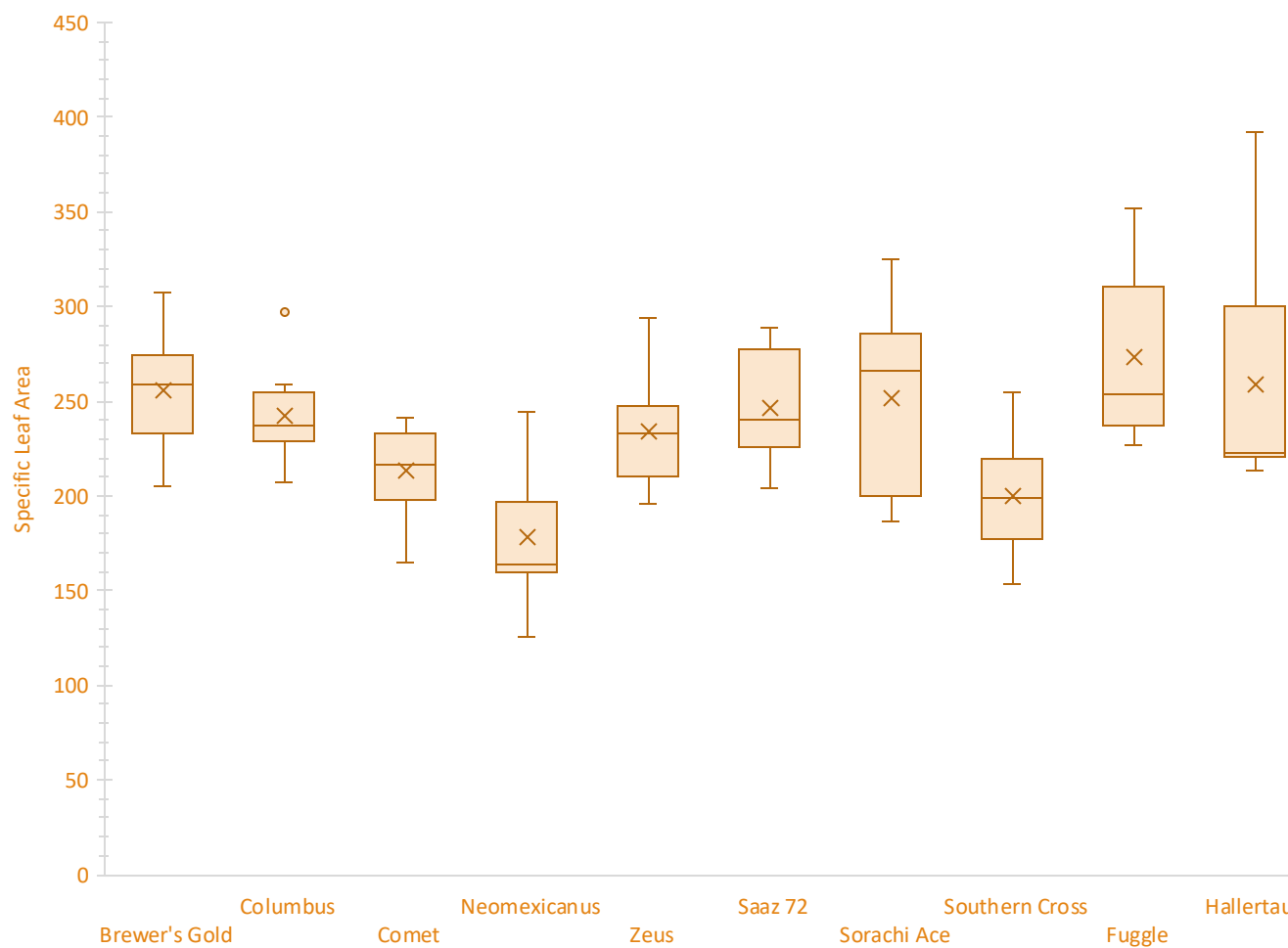
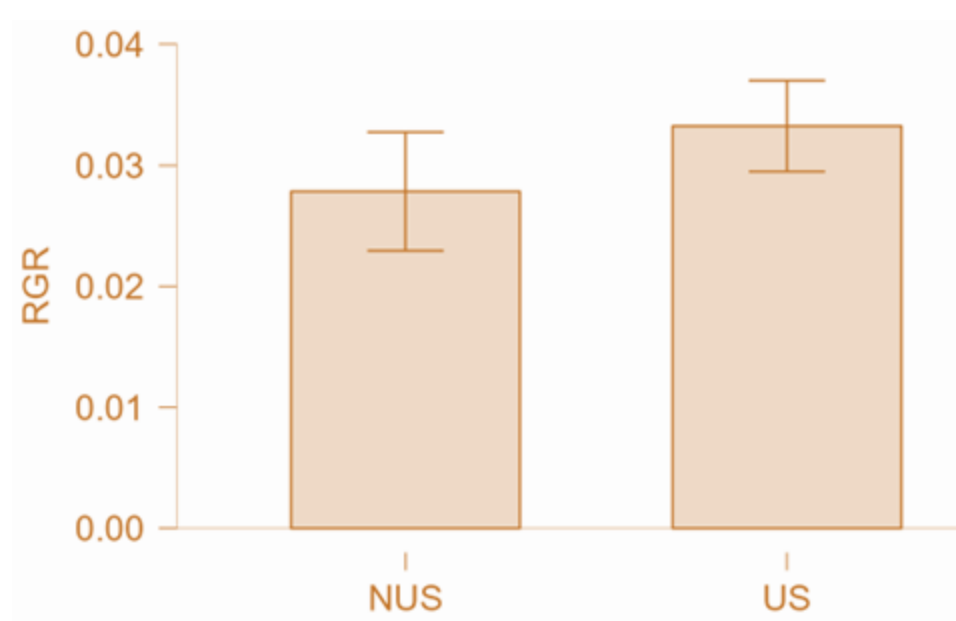


METHODS

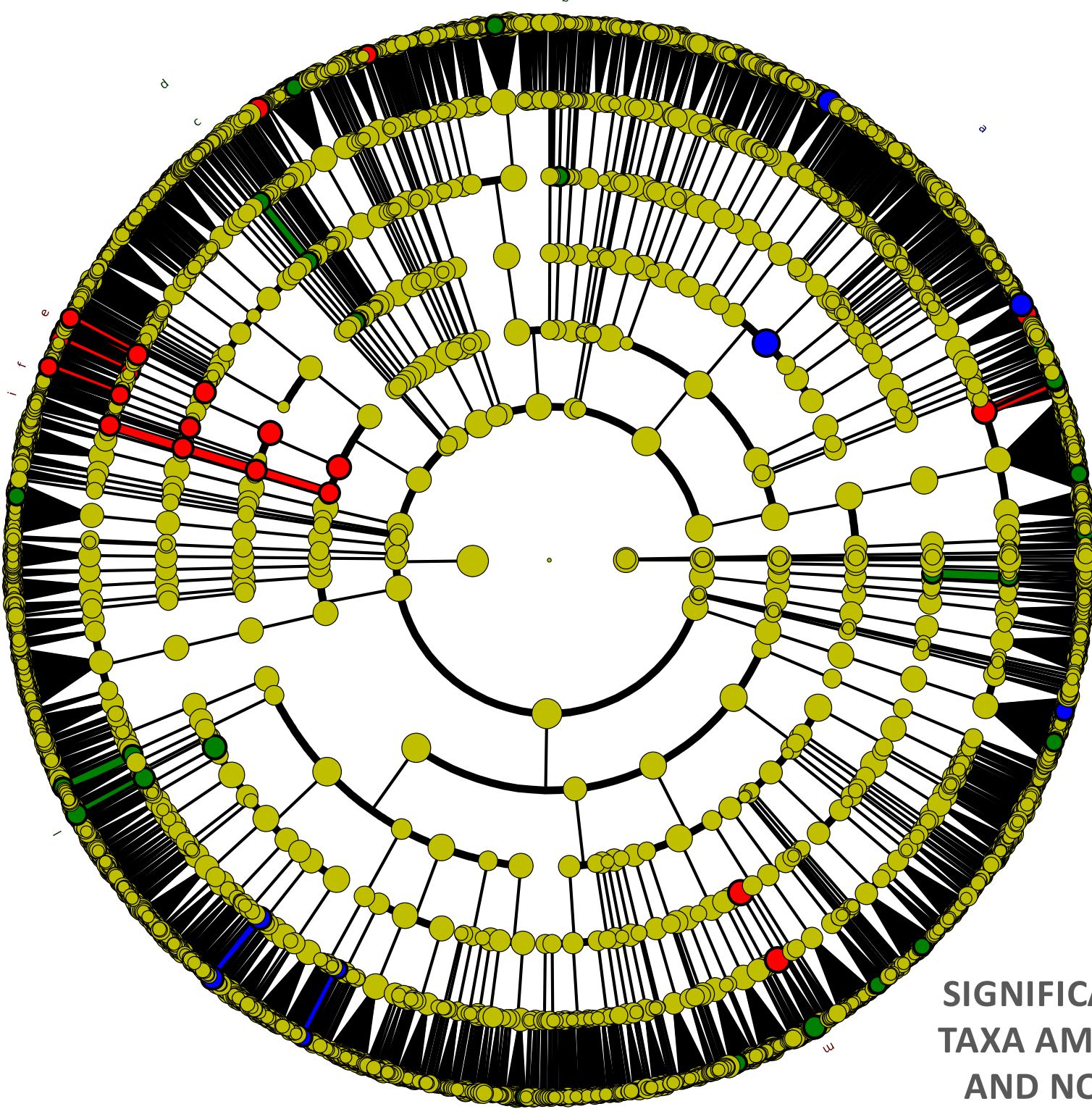
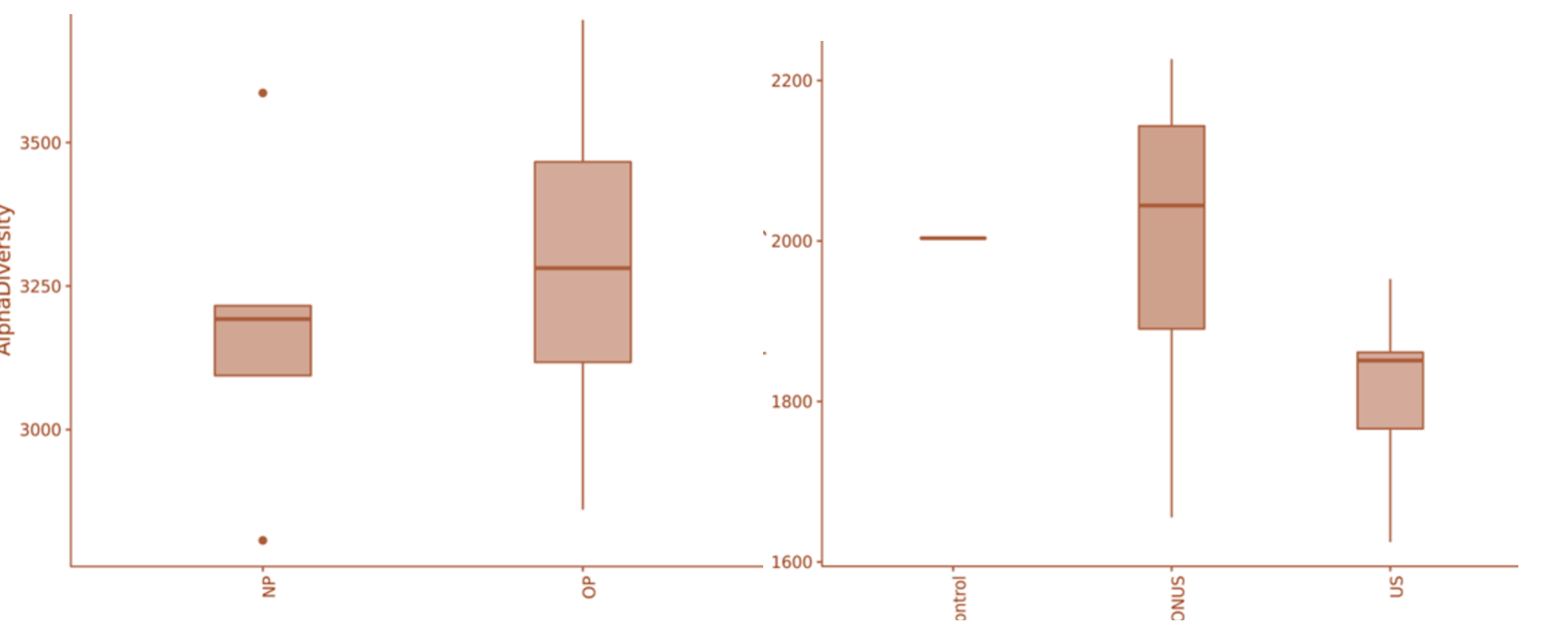
- 10 replicates each of 5 US-based (Brewer's Gold, Columbus, Comet, Neomexicanus, Zeus) and 5 non-US-based (Saaz 72, Sorachi Ace, Fuggle, Southern Cross, Hallertauer) cultivars of hops were established in a common-garden greenhouse experiment in May 2023 and maintained & their growth monitored throughout the growing season.
- Mature leaf samples, and 2 soil core samples, per cultivar were collected at beginning and end of experiment, sampling the top 5 cm, which should contain most soil organic matter, and hence the most microbial, fungal diversity and activity.
- Soil core and mature leaf samples were also collected from multiple replicate hop vines in "new" and "old" plots at Hopportunity Farm in Julian, CA (for comparison with a natural population)
- Soil genomic DNA was extracted from all soil core samples using the ZymoBIOMICS®-96 MagBead DNA Kit, while leaf DNA was extracted using a Macherey Nagel NucleoMag DNA Kit.
- DNA quality, quantity were assessed using gel electrophoresis, nanodrop spectrophotometry, and Qubit Fluorometry.
- 16s rRNA primers (ligated with Illumina adapters) for the V3-V4 region were used to amplify ~500-600 bp from each sample.
- Libraries were uniquely barcoded using Illumina Nextera® XT indices, cleaned, pooled, and analyzed on an Agilent Tapestation®.
- Libraries were sequenced on an Illumina NextSeq 2000™ (150bp, paired-end) at Zymo Research Corp., and data analyzed using the DADA2 (Callahan et al., 2016) + QIIME pipelines (Caporaso et al., 2010)

RESULTS

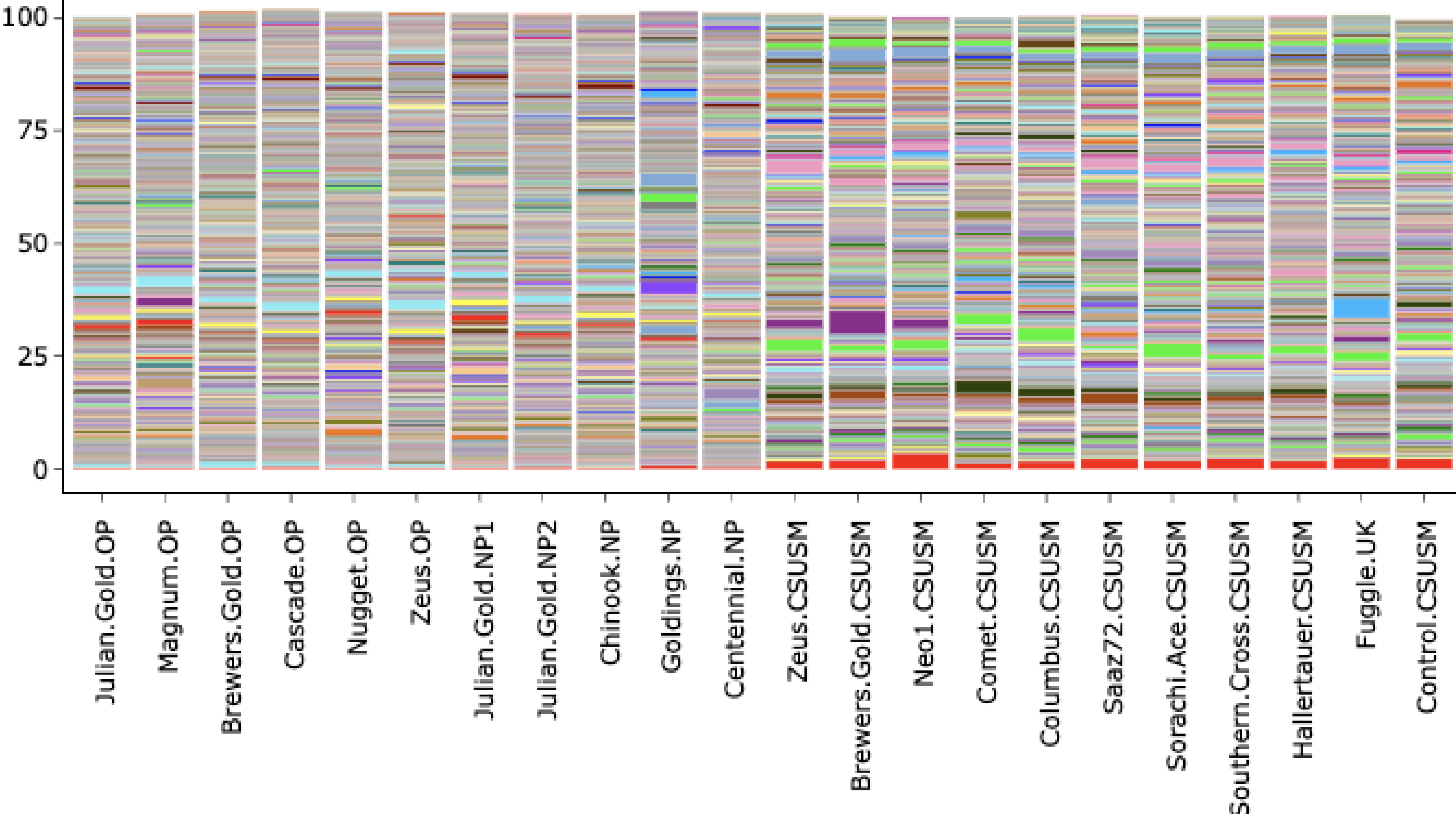
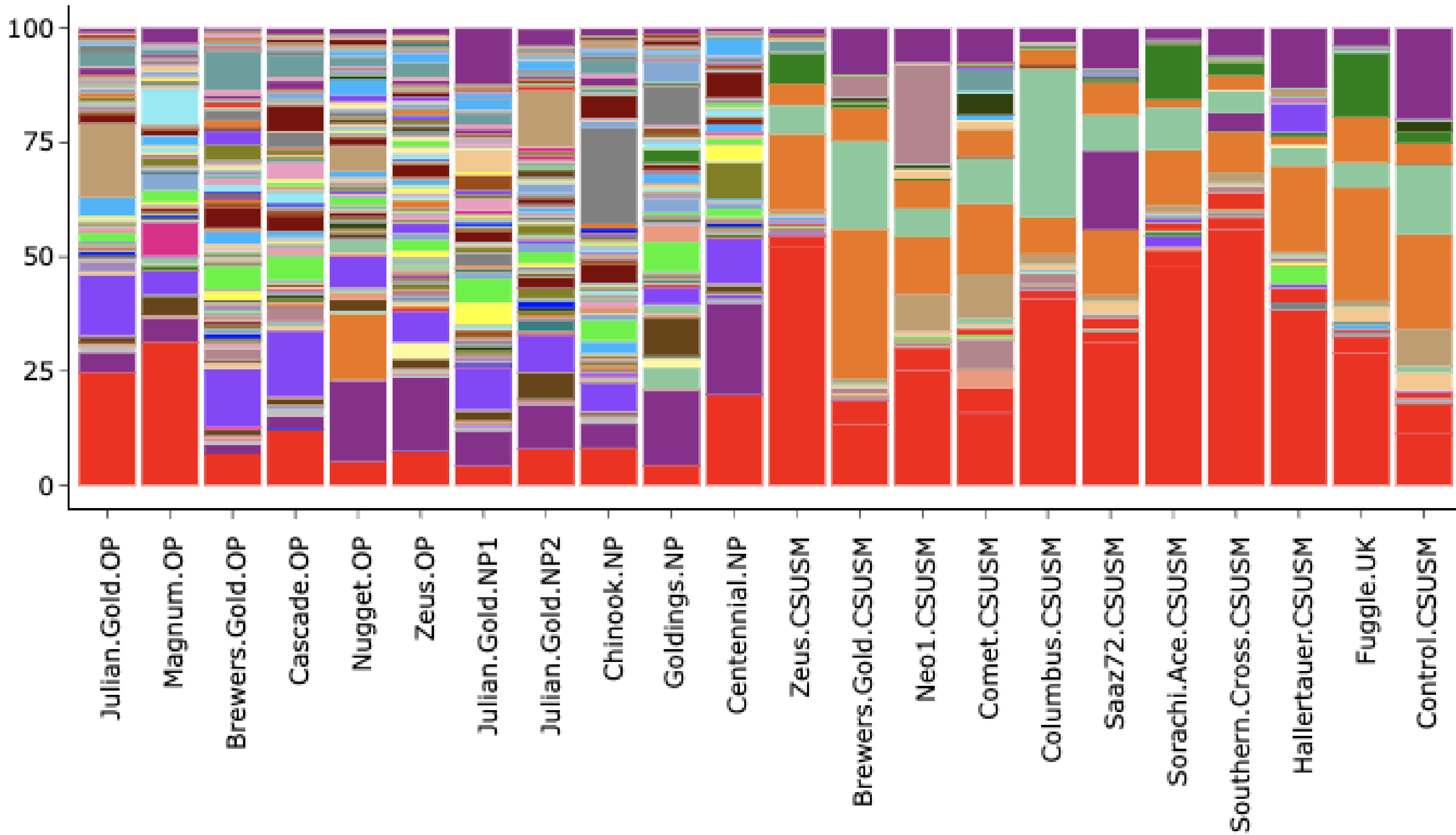
RELATIVE GROWTH RATES (LEFT) AND SPECIFIC LEAF AREAS (RIGHT) ACROSS US AND NON-US BASED CULTIVARS OF HOPS



RHIZOSPHERE BACTERIAL ALPHA DIVERSITIES AT (1) NEW PLOT VS OLD PLOT AT HOPPORTUNITY FARM, JULIAN, CA (LEFT), (2) IN CONTROL SOILS VS NON-US CULTIVARS VS US CULTIVARS AT CSUSM GREENHOUSE



SIGNIFICANTLY DIFFERENT BACTERIAL TAXA AMONGST CONTROL, US-BASED, AND NON-US-BASED CULTIVARS OF HOPS, REPRESENTED AS A CLADOGRAM



FUNGAL (LEFT) AND BACTERIAL (RIGHT) DIVERSITY IN ALL SOIL CORES. OP = OLD PLOT, NP = NEW PLOT, BOTH AT HOPPORTUNITY FARM, JULIAN, CA. CSUSM = SOIL CORES FROM THE COMMON GARDEN EXPERIMENT AT CSU SAN MARCOS GREENHOUSE. NOTE THE SIGNIFICANTLY HIGHER FUNGAL AND BACTERIAL DIVERSITY AT HOPPORTUNITY FARM, COMPARED TO CSUSM, WHICH ALSO SHOWED PRESENCE OF MORE UNCLASSIFIED (SHOWN IN RED).

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- Acosta-Rangel et al. (2021) *Australian Journal of Crop Science* **15.5**: 764–772
- Padgett-Cobb et al. (2023) *Horticulture Research* **10.2**: uhac281
- Callahan et al. (2016) *Nature Methods* **13.7**: 581-583
- Caporaso et al. (2010) *Nature Methods* **7.5**:335-336

