**RestoreNet soil sampling protocol for microbial community composition**

**Labeling:**

1. Label the bag with the [site name]#[plot number] (e.g. MW#14, CRC#7, UT\_PJ#1. See abbreviations and plots list in the table 1) before going to the field. Note: label should be written at the bottom of bag, otherwise it will be covered when the bag is sealed.
2. At the site, record whether any plant is growing or not (G or NG). If legume or other kinds of weed that might influence the microbial community present, also write that down.

**Background/environment screening**

1. Identify the dominant weed in the site. “Dominant weed” are those grass and forbs that are not intentionally introduced into the site but occur in most of the plots. If the hypothesis for this study is that plant species will change the microbial community diversity and composition, then those weeds may also have an influence.
2. Briefly record if any operation (e.g. watering, weeding, fertilization) has been done recently.

**Sampling:**

1. Material: latex/nitrile gloves, sterile plastic sampling bags with wire closure, trowel (with a mark at 5cm depth), bucket, cooler, ice packs, sharpie.
2. Before sampling a new site, rinse the bucket and the trowel with alcohol.
3. Before sampling a new plot, “rinse” the bucket, trowel and the gloves with the soil from the new plot (soil wash), to minimize the contamination from previous site.
4. Collecting three samples across the diagonal of each plot (two corners and one center, see figure 1). Collect soil samples in the middle of two plants, so the root of plants will not be collected.
5. Use trowel to dig a hole about 5cm deep, then transport the loose surface soil to the bucket. Use hands with gloves to mix the three samples from the same plot. Discard rocks, plant roots and other plant material. Collect about 50 to 100 grams of the soil into a sterile plastic bag (figure 2).
6. Roll the bag around the tie several times. Fold the bag horizontally and screw the wire closure tight.
7. Make sure there are in total 21 samples for each of warm sites (2 controls, 10 monocultures, 9 polycultures), 19 samples for cool sites (2 controls, 9 monocultures, 8 polycultures) and 30 samples for each of intermediate sites (2 controls, 16 monocultures, 12 polycultures). Store them in the cooler.

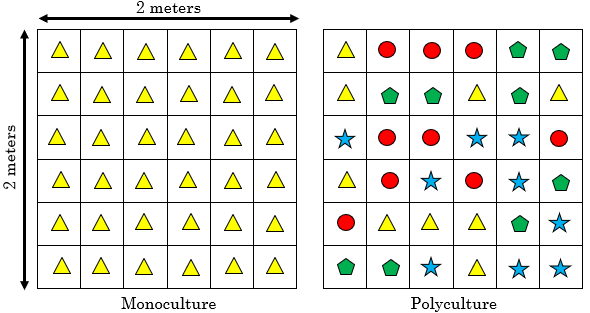
**Storage and transport**

1. Store in a cooler with ice packs before and during the shipping.
2. Do not let samples stay in cooler more than one day without changing the ice packs.



**Figure 1** (left): example of the sampling spots in one plot.

**Figure 2** (right): example of the sampling bag that we use.



**Table 1**: codes/abbreviations for sites and plants.



|  |  |  |
| --- | --- | --- |
| Climate | Scientific name | code |
| IC | *Linum levisii* | LILE |
| *Heliomeris multiflora* | HEMU |
| *Bromus marginatus* | BRMA |
| *Pascopyrum smithii* | PASM |
| *Pleuraphis jamesii* | PLJA |
| *Elymus elymoides* | ELEL |
| IN | *Achillea millefolium* | ACMI |
| *Dalea candida* | DACA |
| *Bouteloua gracilis* | BOGR |
| IW | *Poa secunda* | POSE |
| *Sporobolus cryptandrus* | SPCR |
| *Machaeranthera tanacetifolia* | MATA |
| *Bouteloua eripoda* | BOER |
| *Kranscheninnkovia lanata* | KRLA |
| *Penstemon palmeri* | PEPA |
| *Achnatherum hymenoides* | ACHY |

Only species that occurs in more than one climate setting are selected in this study (study species). IC stands for that plants in this group grow in both intermediate sites and cool sites. IN stands for intermediate group, but they grow in all plots. IW stands for intermediate and warm sites. The order of plants indicates their climate preference (i.e. LILE prefer cool climate most, ACHY prefer warm most among all study species).

**Table 2**: list of monoculture and control plots that plan to be sampled in this study.

Cool sites

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| AZ\_PJ |  |  | CRC |  |  | UT\_PJ |  |  |
| Plot # | type | species 1 | Plot # | type | species 1 | Plot # | type | species 1 |
| 14 | mono | HEMU | 1 | mono | LILE | 1 | mono | PASM |
| 17 | control\_pl | | 3 | mono | PASM | 4 | mono | ELEL |
| 18 | mono | BRMA | 5 | control\_pl | | 6 | mono | BOGR |
| 20 | mono | ELEL | 7 | mono | HEMU | 7 | mono | PLJA |
| 22 | mono | BOGR | 10 | mono | BOGR | 12 | control\_pl | |
| 24 | mono | LILE | 13 | mono | PLJA | 16 | mono | DACA |
| 27 | mono | PASM | 14 | mono | BRMA | 17 | mono | BRMA |
| 30 | mono | ACMI | 19 | control\_pl | | 19 | control\_pl | |
| 31 | control\_pl | | 26 | mono | ACMI | 25 | mono | LILE |
| 32 | mono | PLJA | 29 | mono | ELEL | 31 | mono | ACMI |
| 34 | mono | DACA | 31 | mono | DACA | 36 | mono | HEMU |

Intermediate sites

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| BT |  |  | FM |  |  |
| Plot # | type | species 1 | Plot # | type | species 1 |
| 1 | mono | KRLA | 2 | mono | ELEL |
| 3 | mono | POSE | 4 | mono | DACA |
| 4 | control\_pl | | 5 | control\_pl | |
| 6 | mono | BOGR | 7 | mono | SPCR |
| 9 | mono | PLJA | 9 | mono | LILE |
| 10 | mono | PASM | 11 | mono | ACMI |
| 12 | mono | LILE | 15 | mono | POSE |
| 13 | mono | HEMU | 16 | mono | ACHY |
| 15 | mono | BRMA | 18 | mono | PLJA |
| 17 | mono | SPCR | 20 | mono | MATA |
| 19 | mono | ACMI | 22 | mono | PASM |
| 21 | control\_pl | | 23 | control\_pl | |
| 23 | mono | ELEL | 25 | mono | BOER |
| 25 | mono | MATA | 26 | mono | HEMU |
| 29 | mono | DACA | 28 | mono | KRLA |
| 31 | mono | PEPA | 31 | mono | PEPA |
| 32 | mono | BOER | 33 | mono | BRMA |
| 34 | mono | ACHY | 34 | mono | BOGR |

Warm sites

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| MW |  |  | PF |  |  | SW |  |  |
| Plot # | type | species 1 | Plot # | type | species 1 | Plot # | type | species 1 |
| 6 | mono | KRLA | 2 | mono | POSE | 6 | mono | POSE |
| 8 | control\_pl | | 4 | mono | DACA | 9 | mono | KRLA |
| 9 | mono | PEPA | 7 | mono | KRLA | 11 | mono | PEPA |
| 11 | mono | BOER | 13 | mono | ACMI | 13 | control\_pl | |
| 14 | mono | ACMI | 16 | control\_pl |  | 14 | mono | BOER |
| 17 | mono | ACHY | 17 | mono | MATA | 15 | mono | BOGR |
| 22 | mono | POSE | 19 | mono | PEPA | 21 | mono | ACHY |
| 23 | mono | DACA | 23 | mono | BOER | 22 | mono | MATA |
| 24 | control\_pl | | 26 | mono | ACHY | 27 | mono | SPCR |
| 26 | mono | MATA | 28 | mono | BOGR | 30 | mono | DACA |
| 32 | mono | SPCR | 31 | control\_pl |  | 32 | control\_pl | |
| 33 | mono | BOGR | 36 | mono | SPCR | 33 | mono | ACMI |

Only study species (species that are shared in the sites with different climate setting, see table 1) are selected in this study. Because of the total sample size limit, non-plug control will not be sampled. Table above has been rearranged based on the numerical order of plot number for field work convenience. There are 105 monoculture and control in total.

**Table 3**: list of polyculture plots that plan to be sampled in this study.

Warm sites:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| SW |  |  |  |  |
| 4 | BAMU | BOER | BOGR | POSE |
| 8 | BOCU | BOER | DACA | KRLA |
| 17 | ASTU | BOGR | KRLA | PEPA |
| 18 | ACHY | ACMI | BOER | MATA |
| 19 | ARPU | BOGR | DACA | MATA |
| 20 | MATA | PEPA | SECO | SPCR |
| 23 | ACHY | ARPU | PEPA | POSE |
| 26 | KRLA | PLMU | POSE | SPCR |
| 31 | ACMI | ASTU | DACA | SPCR |
| PF |  |  |  |  |
| 1 | BAMU | BOER | BOGR | POSE |
| 3 | ACMI | ASTU | DACA | SPCR |
| 6 | BOCU | BOER | DACA | KRLA |
| 18 | MATA | PEPA | SECO | SPCR |
| 20 | ASTU | BOGR | KRLA | PEPA |
| 21 | ACHY | ACMI | BOER | MATA |
| 24 | ACHY | ARPU | PEPA | POSE |
| 25 | ARPU | BOGR | DACA | MATA |
| 35 | KRLA | PLMU | POSE | SPCR |
| MW |  |  |  |  |
| 5 | BOCU | BOER | DACA | KRLA |
| 7 | ASTU | BOGR | KRLA | PEPA |
| 10 | ACHY | ACMI | BOER | MATA |
| 15 | ACHY | ARPU | PEPA | POSE |
| 18 | BAMU | BOER | BOGR | POSE |
| 20 | ACMI | ASTU | DACA | SPCR |
| 21 | MATA | PEPA | SECO | SPCR |
| 29 | KRLA | PLMU | POSE | SPCR |
| 34 | ARPU | BOGR | DACA | MATA |

Intermediate sites:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| BT |  |  |  |  |
| 2 | HEMU | LILE | MATA | PLJA |
| 7 | BOGR | HEMU | KRLA | PEPA |
| 11 | KRLA | PLJA | POSE | SPCR |
| 16 | ACMI | ELEL | LILE | POSE |
| 18 | ELEL | MATA | PEPA | SPCR |
| 20 | BOGR | BRMA | DACA | MATA |
| 22 | ACMI | DACA | HEMU | SPCR |
| 24 | ACHY | ACMI | BOER | MATA |
| 27 | ACHY | BRMA | PEPA | POSE |
| 28 | BOER | BOGR | PASM | POSE |
| 30 | BOER | DACA | KRLA | LILE |
| 33 | BOER | BRMA | ELEL | HEMU |
| FM |  |  |  |  |
| 1 | BOER | DACA | KRLA | LILE |
| 3 | HEMU | LILE | MATA | PLJA |
| 6 | ACMI | ELEL | LILE | POSE |
| 8 | KRLA | PLJA | POSE | SPCR |
| 10 | BOGR | HEMU | KRLA | PEPA |
| 12 | ELEL | MATA | PEPA | SPCR |
| 17 | BOGR | BRMA | DACA | MATA |
| 19 | BOER | BRMA | ELEL | HEMU |
| 21 | ACMI | DACA | HEMU | SPCR |
| 24 | BOER | BOGR | PASM | POSE |
| 29 | ACHY | ACMI | BOER | MATA |
| 36 | ACHY | BRMA | PEPA | POSE |

Cool sites

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| AZ\_PJ |  |  |  |  |
| 3 | ACMI | BRMA | LECI | PASM |
| 4 | BOGR | BRMA | DACA | HEBO |
| 9 | ACMI | ELEL | LILE | PSSP |
| 13 | BOGR | ELEL | HECO | PLJA |
| 15 | ACMI | DACA | ELWA | HEMU |
| 19 | HEBO | HEMU | LILE | PLJA |
| 29 | DACA | ELTR | PASM | PLJA |
| 33 | BRMA | ELEL | HEMU | SPGR |
| CRC |  |  |  |  |
| 4 | BOGR | ELEL | HECO | PLJA |
| 6 | BOGR | BRMA | DACA | HEBO |
| 9 | ACMI | BRMA | LECI | PASM |
| 11 | ACMI | DACA | ELWA | HEMU |
| 24 | HEBO | HEMU | LILE | PLJA |
| 27 | DACA | ELTR | PASM | PLJA |
| 30 | ACMI | ELEL | LILE | PSSP |
| 32 | BRMA | ELEL | HEMU | SPGR |
| UT\_PJ |  |  |  |  |
| 2 | BOGR | ELEL | HECO | PLJA |
| 3 | ACMI | ELEL | LILE | PSSP |
| 8 | ACMI | DACA | ELWA | HEMU |
| 20 | BRMA | ELEL | HEMU | SPGR |
| 26 | ACMI | BRMA | LECI | PASM |
| 29 | HEBO | HEMU | LILE | PLJA |
| 30 | DACA | ELTR | PASM | PLJA |
| 35 | BOGR | BRMA | DACA | HEBO |

Polyculture sampling plots are chosen because each of them has at least 3 study species, so we could study the variance between monoculture and polyculture. Species that are colored in orange and blue in warm and cool sites are those species that are not study species.

Plots in the intermediate sites are chosen because they shared three species with plots either in cool sites or warm sites. Species that are colored in green are those shared with cool sites, while that in pink are those shared with warm sites. Species mix at BT#26 and FM#10 are shared with both. In total 75 polyculture plots will be sampled.