

Alex Galang, Jake Coast, George Vourlitis, Elinne Becket  
Molecular Methods in Ecology and Evolution Lab (BIOL 597)

## Introduction

- The CSUSM wetland was built to mitigate for the loss of wetland habitat that occurred from on-campus housing construction in 2002
- Soil environmental variation can alter microbial community composition and virulence factors (VFs)
- This research focused on how vegetation type affects the abundance and profile of virulence factors in rhizosphere soil and bulk soil

## Methods

- Three different vegetation types (lowland, riparian, and sedge) were studied, with two sites (rhizosphere soil and bulk soil) per vegetation type
- Eight soil samples were randomly collected from the upper 20 cm soil layer in each vegetation type
- Bulk soil and rhizosphere soil were separated
- DNA was extracted from all soil samples, followed by shotgun metagenome sequencing
- Using Salmon and the VFDB, abundance of virulence factors in each sample was identified

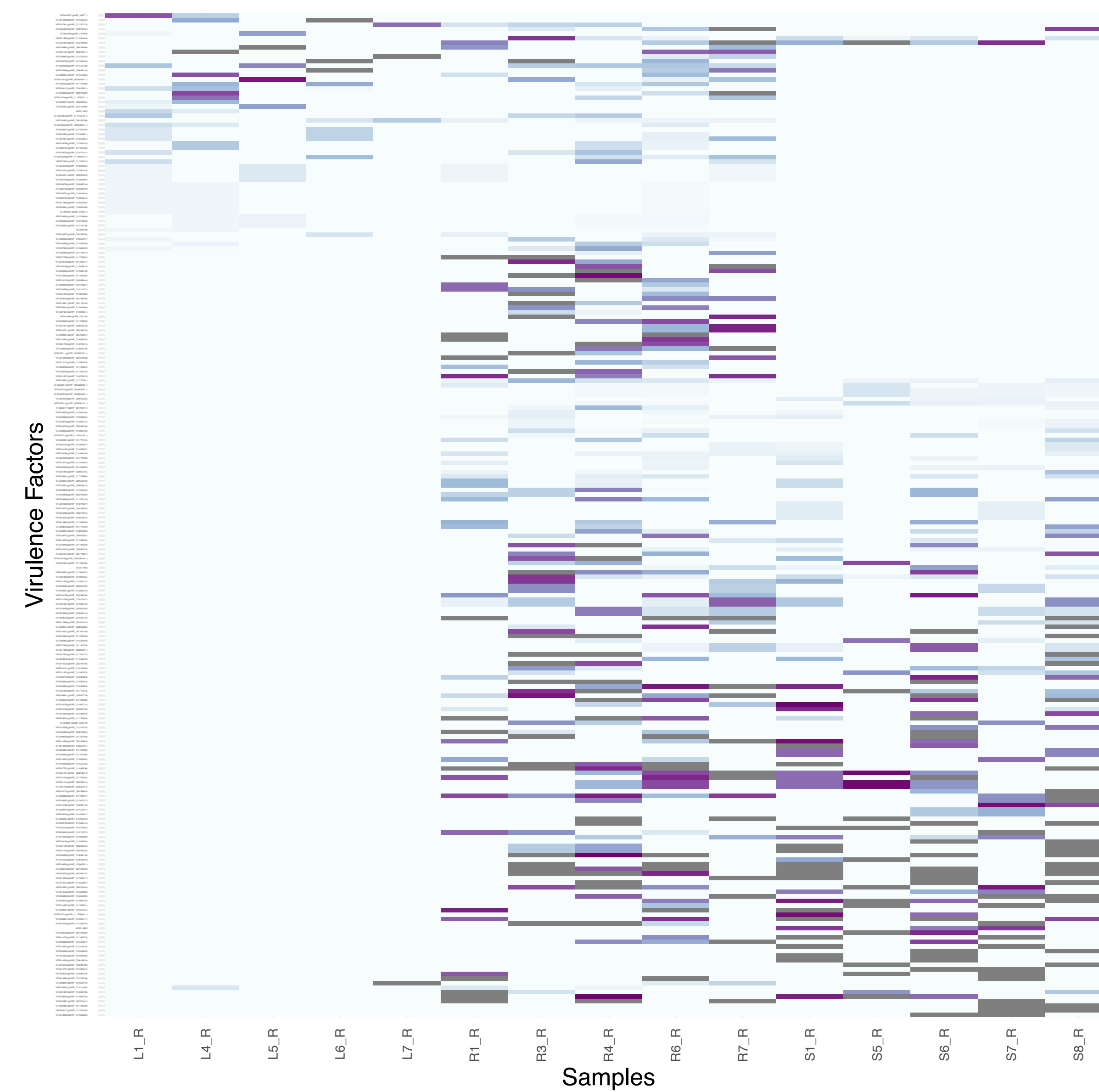
## Conclusions

- Bulk soil had more variability in virulence factors compared to rhizosphere soil
- Differences in virulence factors between the lowland, riparian and sedge can be due to water saturation and anaerobic or aerobic conditions in rhizosphere soil
- The total number of virulence factors across the sampling sites within the rhizosphere soil varied between sample types
- Sedge sites contained VFs common with both lowland and riparian, however lowland and riparian contained more distinct VF profiles in rhizosphere

## Acknowledgements

This research was sponsored by a USDA-NIFA grant to Drs. George Vourlitis and Arun Sethuraman (2018-67032-27701).

## Results and Discussion

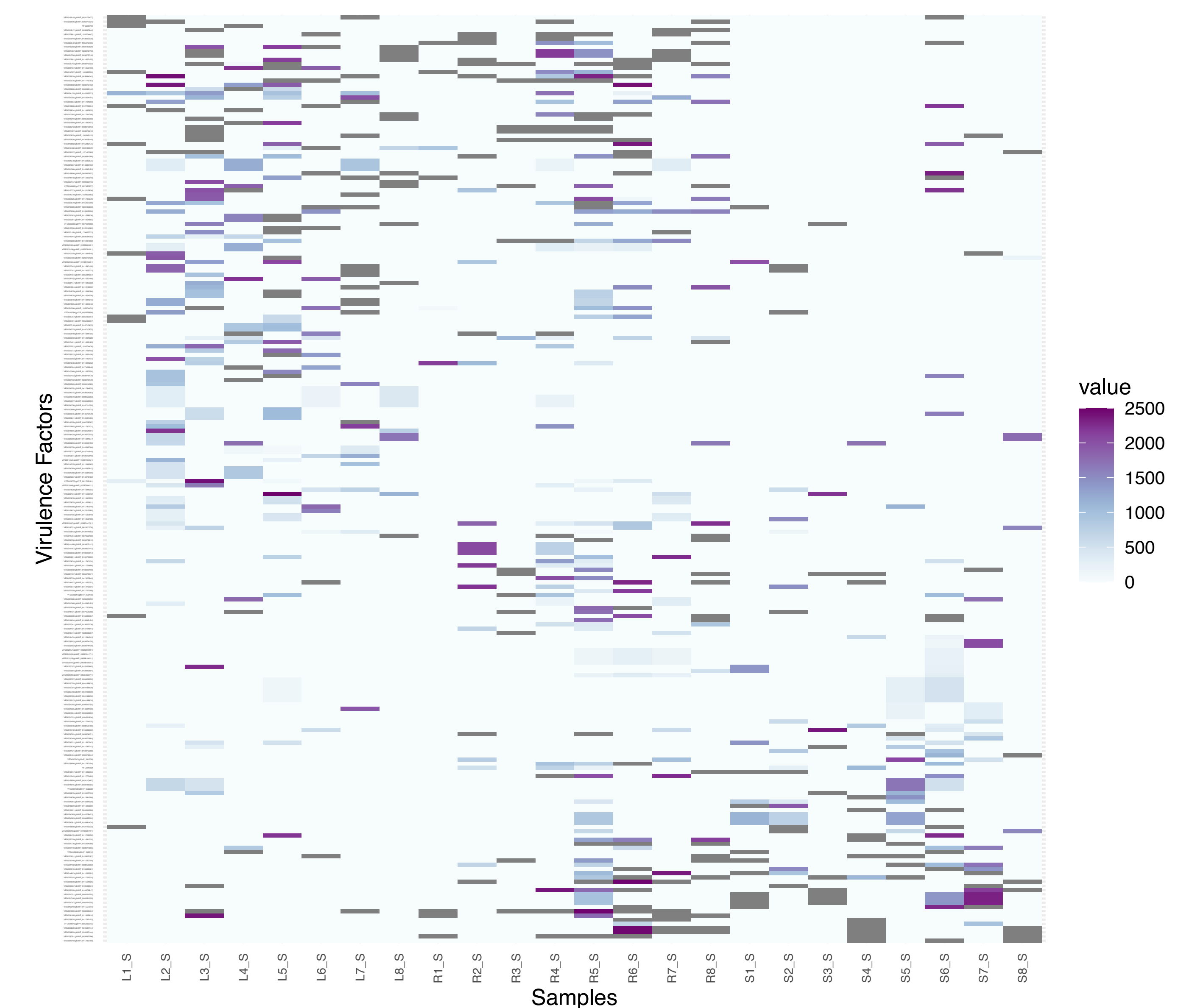


**Fig 1.** Heatmap displaying the relative concentration of virulence factors in the rhizosphere at different sites. L = lowland, R = riparian, S = sedge.

- Rhizosphere soil had less variation in virulence factors than bulk soil (**Fig 1**)
- Lowland and sedge did not share any virulence factors
- Riparian had virulence factors for most differentially abundant pathways

**Table 1.** Virulence factor pathways found in rhizosphere soil across lowland, riparian, and sedge vegetation. Darker color indicates higher abundance.

Virulence Factor Pathway	Lowland	Riparian	Sedge
ESX-1 type VII secretion system EccD1 [ESX-1 (VF0315)]	0	0	810.4
ESX-1 type VII secretion system FtsK/SpolIIE family protein EccCa1 [ESX-1 (VF0315)]	0	476.8	224.4
ESX-1 type VII secretion system FtsK/SpolIIE family protein EccCb1 [ESX-1 (VF0315)]	1084	415.6	0
ESX-5 type VII secretion system EsxA (ESAT-6) homolog [ESX-5 (VF0402)]	0	0	1725.6
ESX-5 type VII secretion system FtsK/SpolIIE family protein EccC5 [ESX-5 (VF0402)]	271.4	0	0
MCE family protein [Mce1 (VF0824)]	0	723.8	0
MCE family protein [Mce2 (VF0825)]	0	1577.2	723
MCE family protein [Mce3 (VF0826)]	0	702	0
MCE family protein [Mce4 (VF0827)]	0	941.8	544.4
MCE family protein [Mce7 (VF0830)]	0	6074.8	883.8
flagellar M-ring protein FliF [Flagella (VF0273)]	0	0	624.6
flagellar basal-body rod protein FlgG [Flagella (VF0273)]	0	730.2	1755.2
flagellar biosynthesis protein FliA [Flagella (VF0273)]	0	402.4	1645.2
flagellar biosynthesis protein FliH [Flagella (VF0273)]	0	1167.6	0
flagellar biosynthesis sigma factor FliA [Flagella (VF0273)]	0	1721.8	1241.8
flagellar biosynthetic protein FliP [Flagella (VF0273)]	0	566.8	2664
flagellar motor protein [Flagella (VF0273)]	0	299.6	3867.6
flagellar motor switch protein FliM [Flagella (VF0273)]	0	1154.8	674
flagellar motor switch protein FliN [Flagella (VF0273)]	0	0	5406.4
flagellar motor switch protein G [Flagella (VF0273)]	0	0	1517
flagellum-specific ATP synthase FliI [Flagella (VF0273)]	0	184.6	2016.8
flagellum-specific ATP synthase FliI [Flagella (VF0430)]	680.4	497.8	0
two-component sensor [Flagella (VF0273)]	0	449.2	297
two-component sensor histidine kinase [SenX3 (VF0857)]	0	787.2	0
two-component sensory transduction protein RegX [RegX3 (VF0858)]	0	1682.2	0
two-component system response regulator PrrA [PrrA/B (VF0823)]	0	2352.4	896.6
type VI secretion system ATPase TsH [HSI-2 (VF0943)]	818.8	801.8	0
type VI secretion system contractile sheath large subunit [HSI-2 (VF0943)]	0	0	841.8
type VI secretion system forkhead-associated protein Fha1 [HSI-1 (VF0334)]	751	0	0
type VI secretion system substrate VgrG1 [HSI-1 (VF0334)]	0	376.2	354.4



**Fig 2.** Heatmap displaying the relative concentration of virulence factors in the bulk soil at different sites. L = lowland, R = riparian, S = sedge.

- Bulk soil had more variation in virulence factors than rhizosphere soil (**Fig 2**)
- Lowland and sedge had more abundance in bulk soil than rhizosphere soil

- In rhizosphere soil, lowland vegetation was less abundant in virulence factors than riparian and sedge (**Table 1**)
- Among the prevalent families of proteins, the total number of virulence factors varied based on site (**Table 2**)

**Table 2.** Total number of virulence factors across all five of each sampled site for four major virulence factor pathways within rhizosphere soil.

Family	Subfamily	Lowland	Riparian	Sedge	
MCE Family	TOTAL	0	55,180	10,756	
	Mce1	0	3,619	0	
	Mce2	0	7,886	3,615	
	Mce3	0	8,592	0	
	Mce4	0	4,709	2,722	
Mce7	0	30,374	4,419		
Flagella-associated protein	TOTAL	0	25,067	65,742	
	FliN	0	0	27,032	
	FliM	0	5,774	3,370	
	G	0	0	7,585	
	FliA	0	8,609	6,209	
	FliP	0	2,834	13,320	
	FliH	0	5,838	0	
	FliA	0	2,012	8,226	
	ESX Type VII Secretion System	TOTAL	6,597	4,462	94,498
		ESX-1 FtsK	5,240	4,462	1,122
ESX-1 EccD1		0	0	4,052	
ESX-5 EsxA		0	0	89,324	
ESX-5 FtsK		1,357	0	0	
Type VI Secretion System	TOTAL	7,849	5,890	5,981	
	ATPase	4,094	4,009	0	
	Contractile sheath (large subunit)	0	0	4,209	
	Fha1	3,755	0	0	
	VgrG1	0	1,881	1,772	