The metagenomic signatures of impacted environments: Unravelling the microbial community dynamics in ecosystem function

Renee J. Smith

BSc Hons



Thesis submitted for the degree of Doctor of Philosophy

September 2012

School of Biological Sciences
Flinders University
Adelaide, Australia

Table of Contents

Sum	ımar	y	V
Ack	now	edgements	. v i
Dec	larat	ion	vi
Cha	pter	1	
Gen	eral	Introduction	1
1.1	Mi	crobial communities run the world	2
1.2	Mi	crobial communities as biological indicators	3
1.3	An	thropogenic disturbances	4
1.4	Th	esis Objective	6
1.5	Th	esis Structure	7
Cha	pter	2	
Meta	_	mic comparison of microbial communities inhabiting confined and	
2.0		confined aquifer ecosystems	
2.0		mmary	
2.1		roduction	
2.2	Re	sults	13
2	.2.1	Overview of the biogeochemical environment and microbial enumeration	12
2	2.2		
		Taxonomic and metabolic profiling of groundwater metagenomes	
	.2.3	Comparison of metabolic and taxonomic profiles from other habitats.	
2.3		cussion	
	.3.1	Aquifer systems	
	.3.2	Taxonomic profiling of groundwater	
	.3.3	Metabolic profiling of groundwater	
	.3.4	Comparison to other microbial communities	
	.3.5	Caveats	
2.4		nclusion	
2.5	$\mathbf{E}\mathbf{x}$	perimental Procedures	26

2	2.5.1	Site selection	26
2	2.5.2	Sampling Groundwater	28
4	2.5.3	Microbial enumeration	30
2	2.5.4	Sample filtration, microbial community DNA extraction and sequencing	30
2	2.5.5	Data analysis	31
2.6	Ac	knowledgements	32
Ch	apter	3	
Co	nfine	l aquifers as viral reservoirs	41
3.0	Su	mmary	42
3.1	Int	roduction	43
3.2	Re	sults and Discussion	44
3.3	Ac	knowledgements	49
		4 hydrocarbon impacts on the structure and functionality of meeshore microbial communities: A metagenomic analysis	
4.0		stract	
4.1		roduction	
4.2		terials and Methods	
4	4.2.1	Site selection and sampling	58
4	4.2.2	Extraction and quantification of hydrocarbon	58
2	4.2.3	Nutrient analysis, microbial community DNA extraction and sequencing for metagenomic analysis	60
4	4.2.4	Data analysis	60
4.3	Re	sults	62
4	4.3.1	Nutrient and hydrocarbon analysis	62
4	4.3.2	Taxonomic and metabolic profiling of beach metagenomes	
4.4	Dis	cussion	67
4.5	Ac	knowledgements	75

Cha	pter 5				
Dete	ermining the metabolic footprints of hydrocarbon degradation usi	ng			
	multivariate analysis	85			
5.0	Abstract	86			
5.1	Introduction	87			
5.2	Materials and Methods	89			
5.	.2.1 Data collection	89			
5.	.2.2 Data analysis	89			
5.3	Results	91			
5.4	Discussion	92			
5.5	Conclusion	97			
5.6	Acknowledgements	97			
Cha	pter 6				
Tow	vards elucidating the metagenomic signature for impacted				
	environments	103			
6.0	Abstract	104			
6.1	Introduction	105			
6.2	Materials and Methods	107			
6.	.2.1 Data collection	107			
6.	.2.2 Data analysis	108			
6.3	Results	110			
6.4	Discussion	114			
6.5	Conclusion	117			
6.6	Acknowledgements	118			
	Chapter 7				

7.1.1	Metagenomics comparison of microbial communities inhabiting confined and unconfined aquifer ecosystems	124
7.1.2	Confined aquifers as viral reservoirs	125
7.1.3	Effect of hydrocarbon impacts on the structure and functionality of marine foreshore microbial communities: A metagenomic analysis	127
7.1.4	Determining the metabolic footprints of hydrocarbon degradation using multivariate analysis	_
7.1.5	Towards elucidating the metagenomic signature for impacted environments	129
	esis Synthesis: Demonstration of microbial indicators for impacted vironments	
Reference	ces	133
Appendi	ix 1	
Pul	hlished manuscrints arising from and related to this thesis	175

Summary

Microbes are largely responsible for the turnover of energy and matter and are thus, integral players in ecosystem functioning. Despite the increasing awareness of the importance of microbial communities, there is still a critical lack of information on the complex relationship between microbial communities and the environment. Metagenomic analysis is thought to yield the most quantitative and accurate view of the microbial world, greatly increasing our ability to generate microbial profiles of the changing world. These methodologies have led to the growing interest in understanding and forecasting microbial responses to anthropogenic disturbances. This thesis investigates the microbial responses to two common forms of pollution, agricultural modification and hydrocarbon impact, to determine to what extent the resident microbial communities may be effected by introduced contaminants. The reoccurring theme of this thesis has been that major shifts in the structure and function of the resident microbial communities was observed following environmental change. Moreover, this thesis demonstrated that the microbial communities inhabiting impacted environments exhibited markedly different community responses based on contaminant type, allowing for the discrimination of their metagenomic signatures. This thesis provides detailed insight into how environmental change affects the inhabiting microbial consortia, and for the first time, demonstrates how the overall metagenomic signature can be used to detect and assess the extent to which anthropogenic disturbances have altered our planet.

Acknowledgements

I would firstly like that thank my primary supervisor Assoc. Prof. Jim Mitchell for his mentoring, guidance and support throughout this project. Thank you for believing in me (even when I didn't), the humour that kept me sane and above all else, the opportunities that allowed me to be the best that I could be. I truly appreciate everything you have done for me. I would also like to thank my co-supervisor Assoc. Prof. Melissa Brown for your encouragement and advice over the course of this study.

A big thanks to the 'Mitchell-Seuront Lab' over the years, in particular to Tom Jeffries, your endless patience and assistance throughout my time in this lab has helped me more than you could possibly know. To Kelly Newton, Ben Roudnew, Justin Seymour and James Paterson, thank-you for your advice, training, support and friendship from the start of this project. I would also like to thank members of the Evolutionary Biology Unit, in particular Alison Fitch who has been a great source of information and support during the many, many hours of trial and errors in the lab.

Also thank you to my friends and family, in particular to my parents, who have been a constant source of inspiration for me. I would not be where I am today if it was not for your constant love, support and encouragement, and for that I am forever grateful.

And last but not least I would like to thank my partner Michael. You have been amazing throughout the course of this PhD and I am constantly surprised by your generosity, humour, friendship and love. Without you I could not have come this far.

Declaration

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Renee Jade Smith

September 2012