

What Drives Bacterial Community Structure in Stream Biofilms?



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BACKGROUND

The microorganisms within biofilms are the key basal trophic level within most freshwater systems. However, microbial structure, function and succession in natural stream systems remain poorly understood. This research characterises the biofilm community structure of stream biofilms experiencing different anthropogenic impacts and how they change over time.

Our aim is describe the changes in bacterial biofilm communities over time and to investigate what drives these changes.

CONCLUSIONS

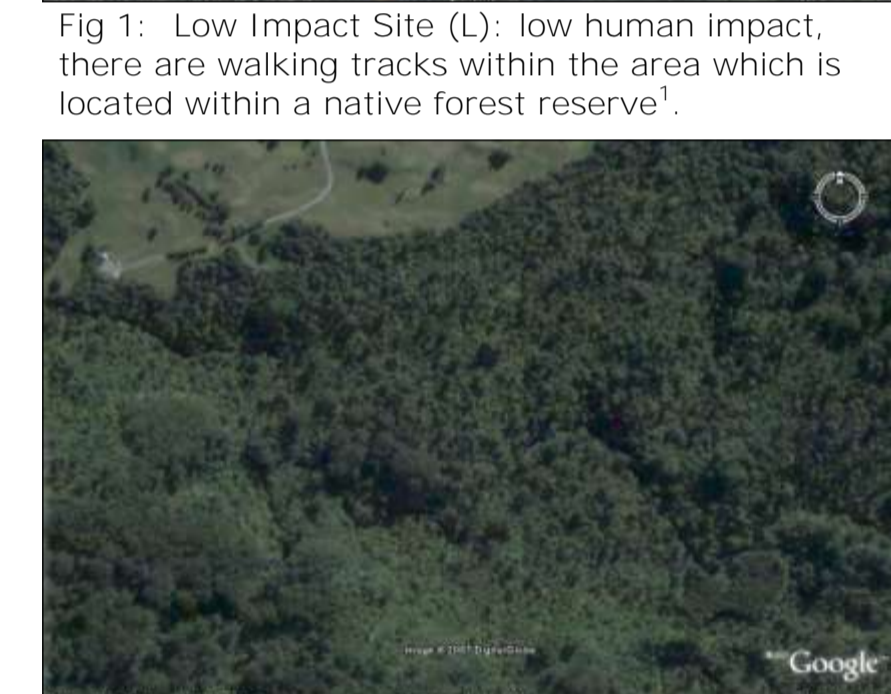
- Population structure is different for each of the four sites at both class and genus level.
- Season is an important driver of bacterial community structure.
- Gammaproteobacteria* and *Alphaproteobacteria* are the most variable populations.
- Anthropogenic impact is an important driver of bacterial community structure.
- Total inorganic nitrogen and dissolved oxygen explain ~ 40 % of variability in bacterial community structure.
- 60% of variability is unexplained by parameters tested.

RESEARCH STRATEGY

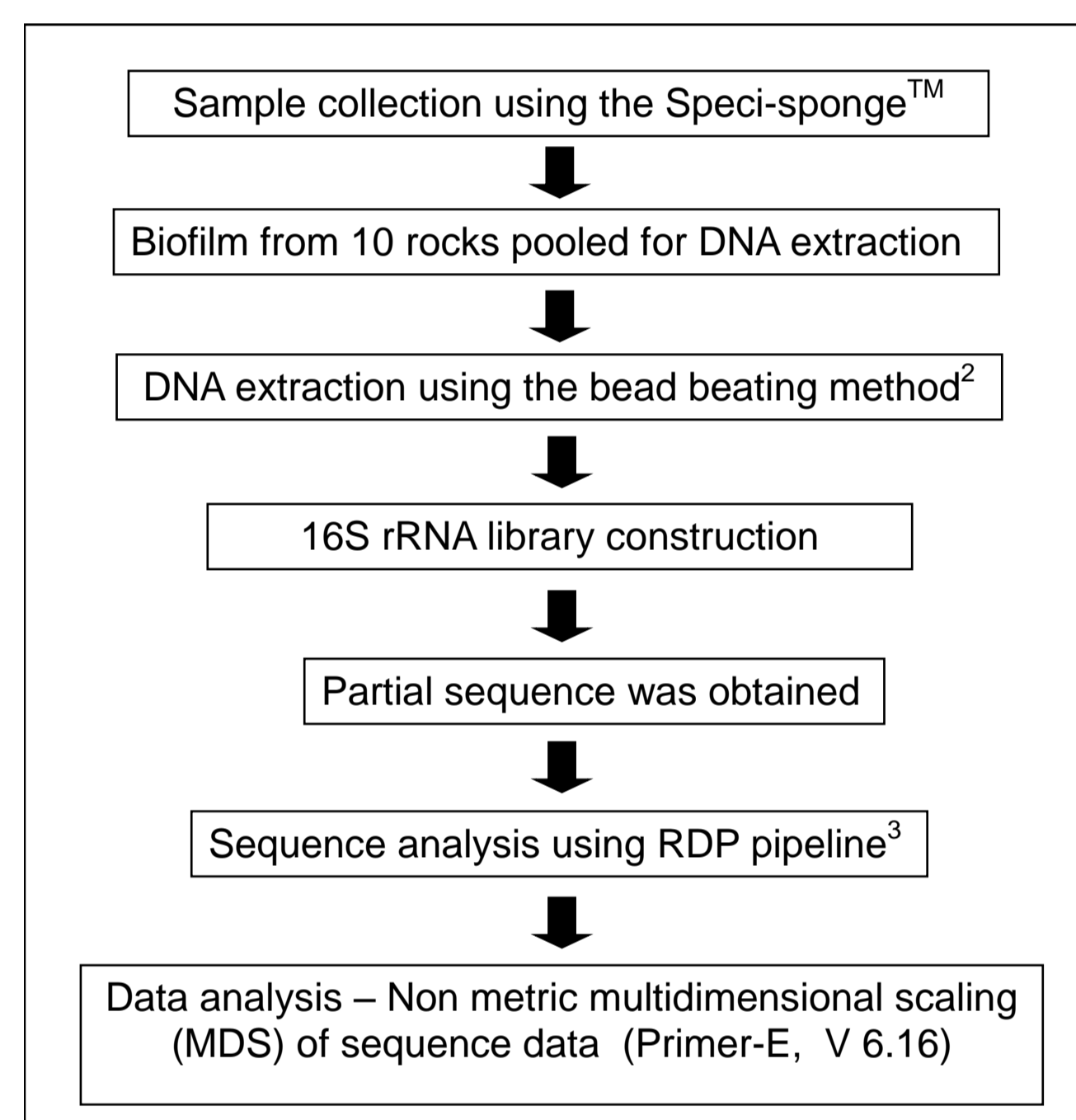


SITE SELECTION

Four stream sites were selected within the Auckland region (New Zealand). These represent different levels of impact based on surrounding catchment use, and range from a low impact stream within a native forest to a highly modified urban stream (Fig 1-4). Seasonal samples of stream biofilms were collected over a two year period from September 2004 to July 2006.



METHODOLOGY OVERVIEW



PHYLOGENETIC CLASSIFICATION AND DATA MANIPULATION

- Assignment of classification was set at a confidence value of 95% in RDP pipeline.
- Sequences were chimera checked using Mallard⁴
- A sequence was classified to a phylogenetic level when the percentage match was >95% (Table 1).
- The sample data was standardised so that each taxonomic unit represented a proportion of the total number of clones obtained for each sampling date and site.



Order	Order %	Family	Family %	Genus	Genus %	Final Classification
Enterobacteriales	100%	Enterobacteriaceae	100%	Salmonella	56%	Unclassified Enterobacteriaceae
Burkholderiales	100%	Comamonadaceae	100%	Acidovorax	100%	Acidovorax

Table 1: An example of phylogenetic assignment from the low-medium impact site for September 2004.

RESULTS

A total of 32 clone libraries were generated with 3200 quality sequence generated. These were used for subsequent analysis and phylogenetic assignment (Fig 5 - 10).

- 27 classes (Fig 5) and 360 genus were represented over the 8 seasons.
- Gammaproteobacteria* were generally the dominant class for Low to medium impact sites.
- Cyanobacteria* were the dominant class for high impact site

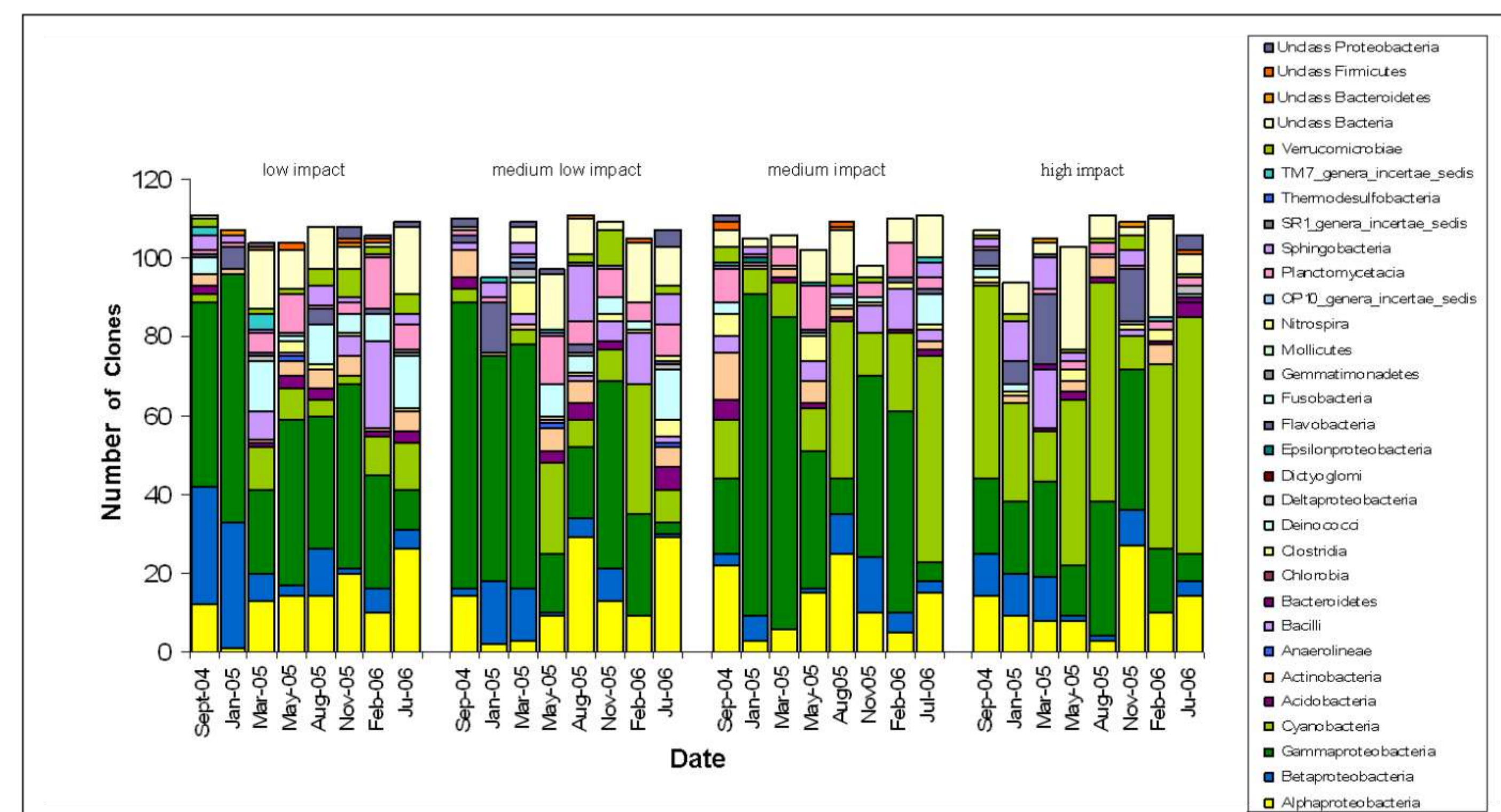


Fig 5: Stream Biofilm Community profile from clone library data for sites experiencing Low (L), Medium Low (ML), medium (M) and high (H) anthropogenic impact from September 2004 to July 2006.

RESULTS

WHAT IS THE RELATIONSHIP BETWEEN BACTERIAL CLASS AND SEASONS?

The four sites were compared by conducting non-metric multidimensional scaling (MDS) of all sites over the two year sampling period (Fig 6, 7). MDS compares the variability between organism class (or genus), frequency of occurrence and site. The relationship of these three factors is depicted in a multi-dimensional plot.

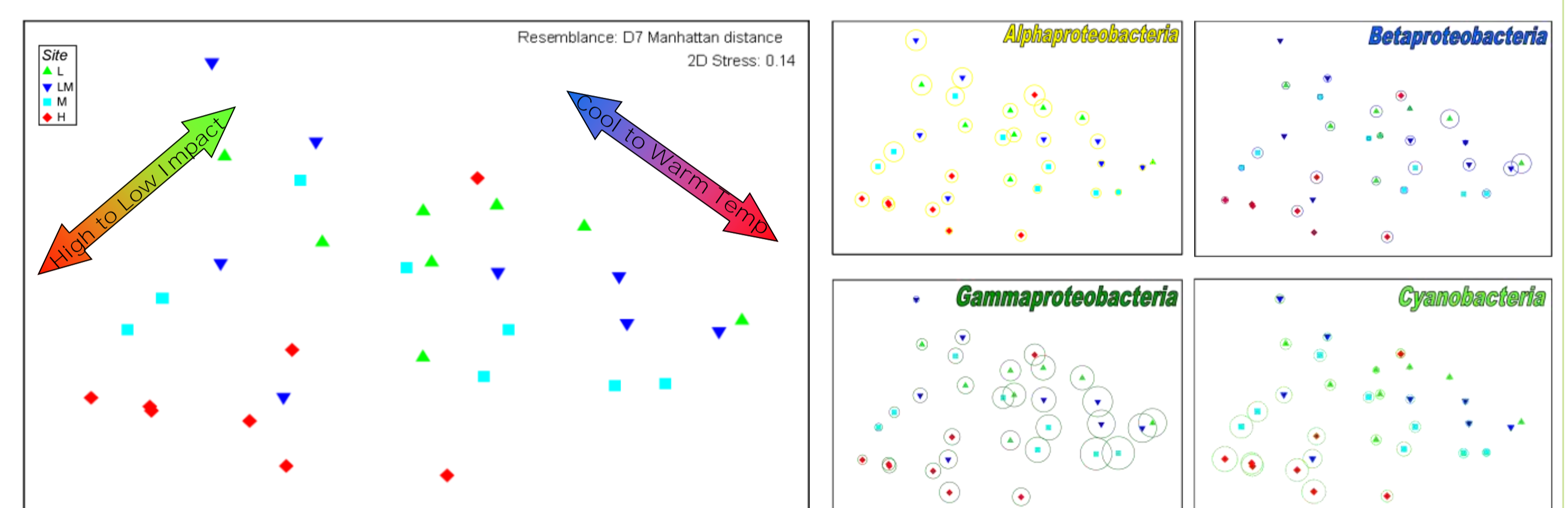


Fig 6: MDS ordination of Class Level abundance data for the Low, Medium Low, Medium and High impact sites from composite biofilm samples from Sept 2004 to July 2006. The plots labeled *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria* and *Cyanobacteria* show bubbles where these classes were present. The larger the bubble the greater the proportion of that class within that samples population.

- Seasonal fluctuations in population are evident between the *Alphaproteobacteria* (increasing in cooler months) and *Gammaproteobacteria* (increasing in warmer months).
- Betaproteobacteria* are generally present regardless of site or date and could indicate a relatively stable population in stream biofilms.
- Cyanobacteria* dominate in the high impact site over 7 seasons (clustering at the lower left hand corner) and increase at other sites during warmer months
- The high impact site is not dominated by *Cyanobacteria* in Nov 05 possibly due to high rainfall and flow in the two weeks preceding sampling. This may have scoured the upper filamentous part of biofilm (*Cyanobacteria*) and exposed the underlying biofilm.

TRACKING BACTERIAL BIOFILM COMMUNITY STRUCTURE OVER TIME (GENUS LEVEL)

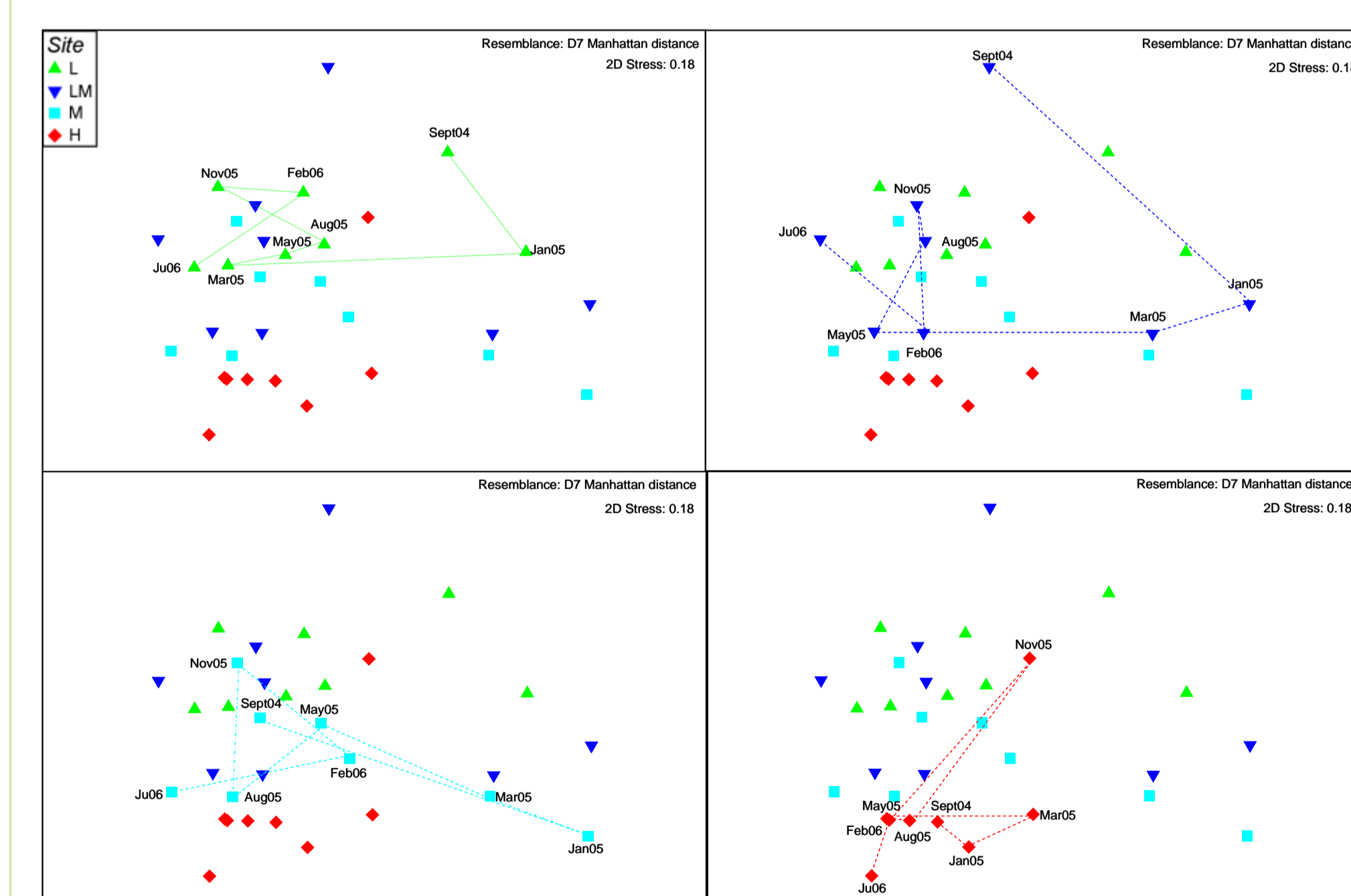


Fig 7: MDS ordination of Genus Level abundance data for the Low, Medium Low, Medium and High impact sites from composite biofilm samples from Sept 2004 to July 2006.

- Populations change in a broadly consistent way with early samplings to the right of the plot and later ones to the left.
- Low to medium impact sites show major shifts in populations between Sept-May.
- The high impact site populations are generally localised in the lower left hand part of the MDS plot with a major shift evident in Nov 05.

EFFECT OF ENVIRONMENTAL PARAMETERS ON BIOFILM COMMUNITY STRUCTURE

The low and high impact sites were selected to evaluate the contribution of environmental parameters to changes in community structure for the 2005 sampling sessions. A resemblance matrix of genus level abundance was generated using Manhattan distance similarity measure and compared to the environmental data using the program Distal-M⁵ (Table 2). The variables included were pH, water temperature, turbidity, suspended solids, dissolved oxygen, ammoniacal nitrogen, total inorganic oxidised nitrogen, total Kjeldhal nitrogen, dissolved reactive phosphorus, total phosphorus, monthly average air temperature, average monthly rainfall and average monthly radiation. Significant results are shown in Table 2.

Variable	SS (Trace)	pseudo-F	P	Prop	Cumulative
Total Inorganic Nitrogen	1.6820	2.3024	0.0062	0.2235	0.2235
Dissolved Oxygen	1.2599	1.9238	0.0407	0.1674	0.3909

Table 2: Significant environmental parameters that influence bacterial biofilm populations.

- Total inorganic nitrogen accounts for 22.3 % of the variation in stream biofilm communities.
- Dissolved oxygen accounts for 16.7 % of the variation in stream biofilm communities.
- 60% of the variation is not explained by the environmental variables tested.
- Some other influential variables not measured could include predation by protozoa and macro-invertebrates, organic carbon availability and within biofilm microbial loop processes.

References
1. Aerial photos from Google Earth available from www.earth.google.com
2. Miller, D.N., J.E. Bryant, E.L. Madsen, W.C. Ghorse (1999). Evaluation and optimisation of DNA extraction from Soil and Sediment Samples AEM. 65 (11): 4715-4724.
3. Wang, Q. G. M. Garrity, J. M. Tiedje, and J. R. Cole (2007). Naive Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. AEM. 73(16):5261-7
4. Ashelford, K.E., N.A. Chuzhanova, J.C. Fry, A.J. Jones and A.J. Weightman (2006) New Screening Software Shows that Most Recent Large 16S rRNA Gene Clone Libraries Contain Chimeras. AEM 72(9): 2734-41.
5. Marti Anderson Distal-M programme <http://www.stat.auckland.ac.nz/~mja/Programs.htm#Mine>