

Microbial Indicators of Faecal Contamination of Groundwater after Application of Municipal Biosolids

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Goals:

- Assess the risk to water resources associated with land application of municipal biosolids
- Evaluate the best indicator for microbial contamination stemming from biosolids
- Determine the potential of management practices to mitigate the risk for off-site transport of biosolids microbial contaminants

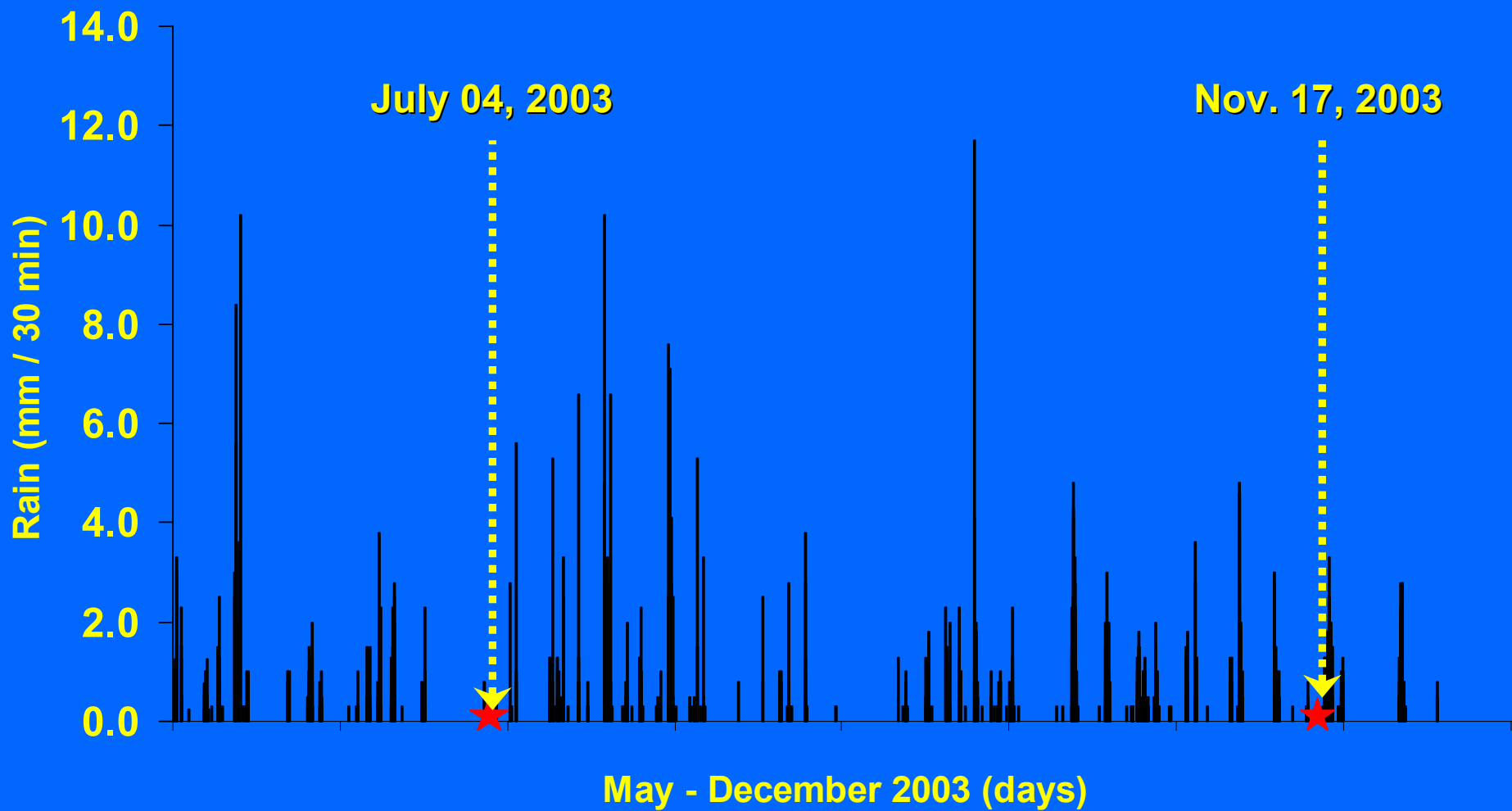
Objectives:

1. Develop prediction tools for identifying soil properties conducive to initiating preferential flow
2. Identify possible management tools to minimize the potential for preferential transport of pathogens from biosolids to water resources
3. Develop microbiological means to quantify microbial transport associated with the land application of the sewage biosolids
4. Evaluate possible management practices for reducing preferential transport of bacteria after the land application of the sewage biosolids

- **Field experimentation – plot level:**
 - **Drainage patterns**
 - **Microbial indicator evaluation –relevance and persistence**
 - **Management practices (biosolid type, tillage, seasonality)**
- **Laboratory testing – undisturbed soil cores (45cm diam. X 50 cm depth & 10cm diam. X 20cm depth)**
 - **Drainage patterns**
 - **Microbial & particulate transport – spatial and temporal patterns**
 - **Microbial persistence**
 - **Evaluation of significance of soil properties**
 - **Evaluation of possible impact of surface charge modifiers (i.e. surfactant used for dewatering municipal biosolids)**

Field experimentation

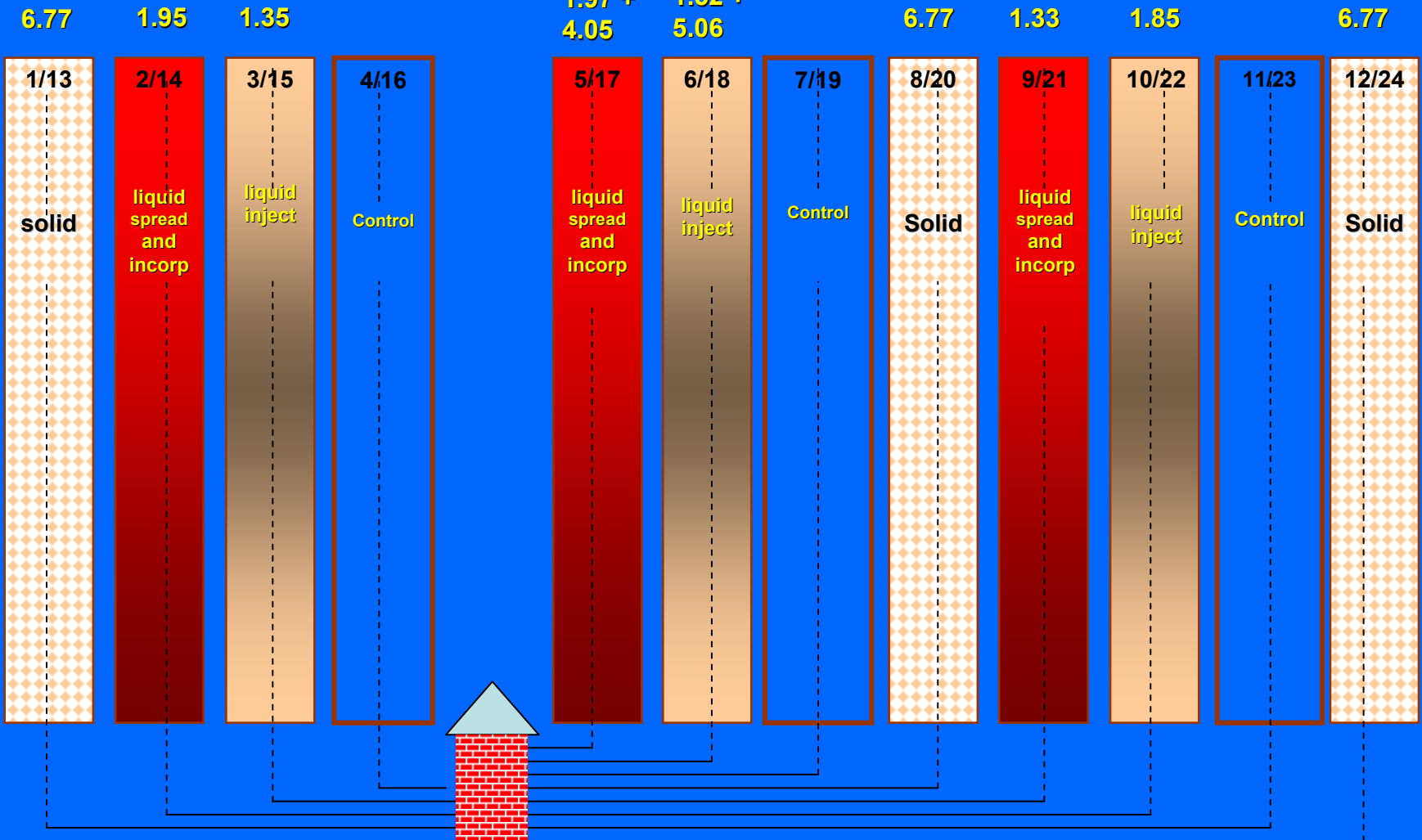
Hydrologically isolated plots – land application of biosolids Alfred College, 2003



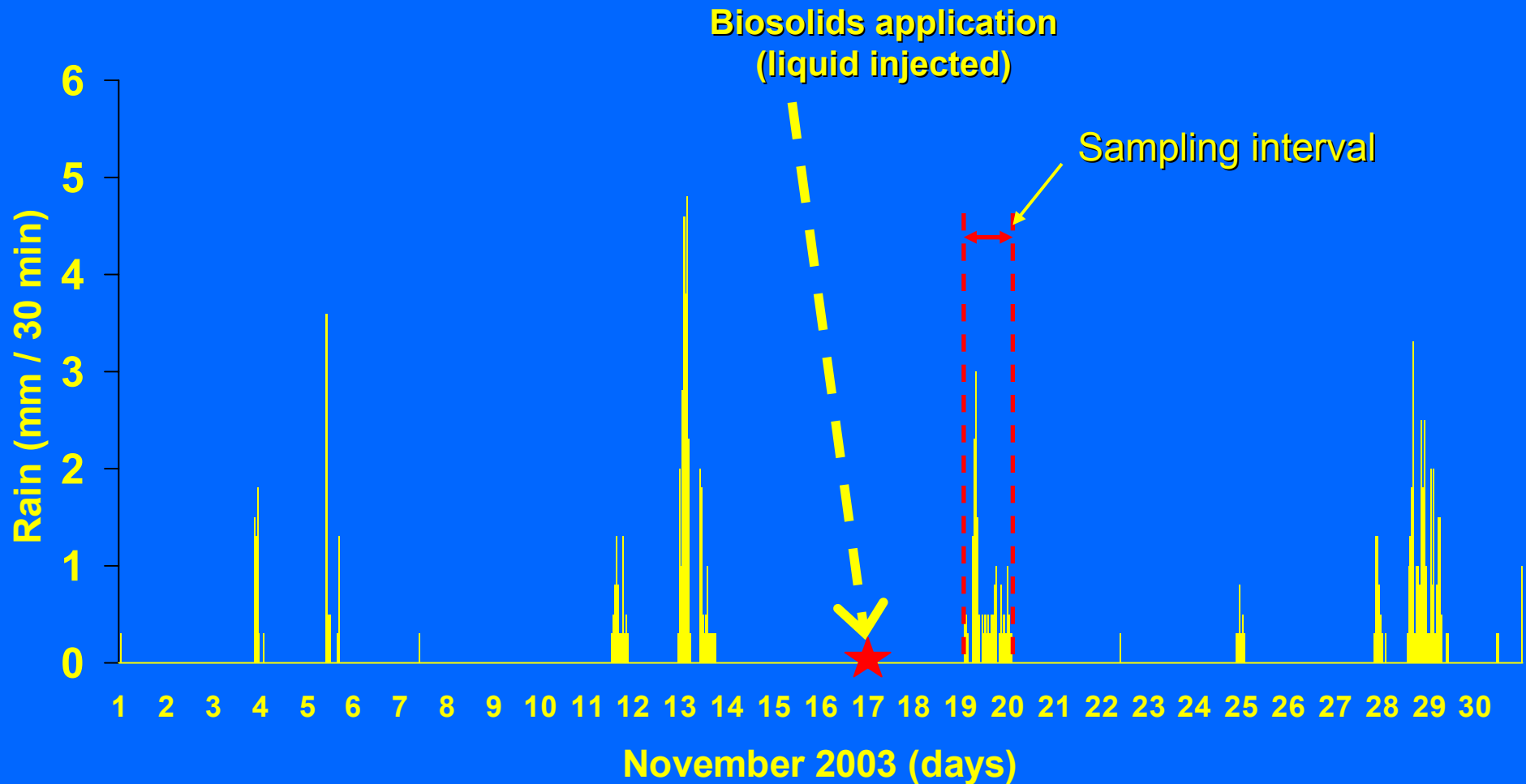
Hydrologically isolated plots – Land application of biosolids - 2003

TDR Instrumented

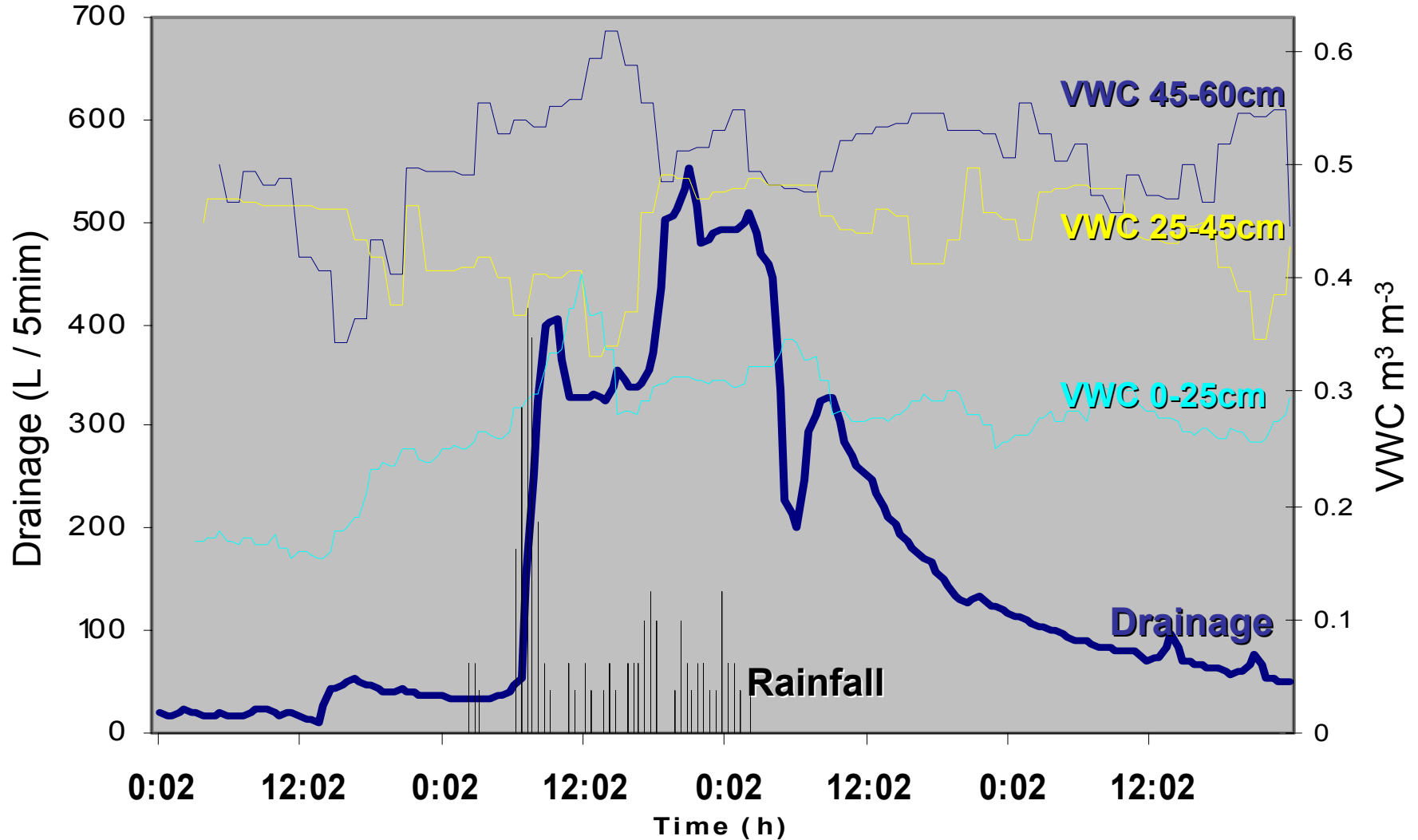
Dry matter applied (tonne/ha⁻¹):



**e.g. Fall application of biosolids
Liquid injected – November 17, 2003**



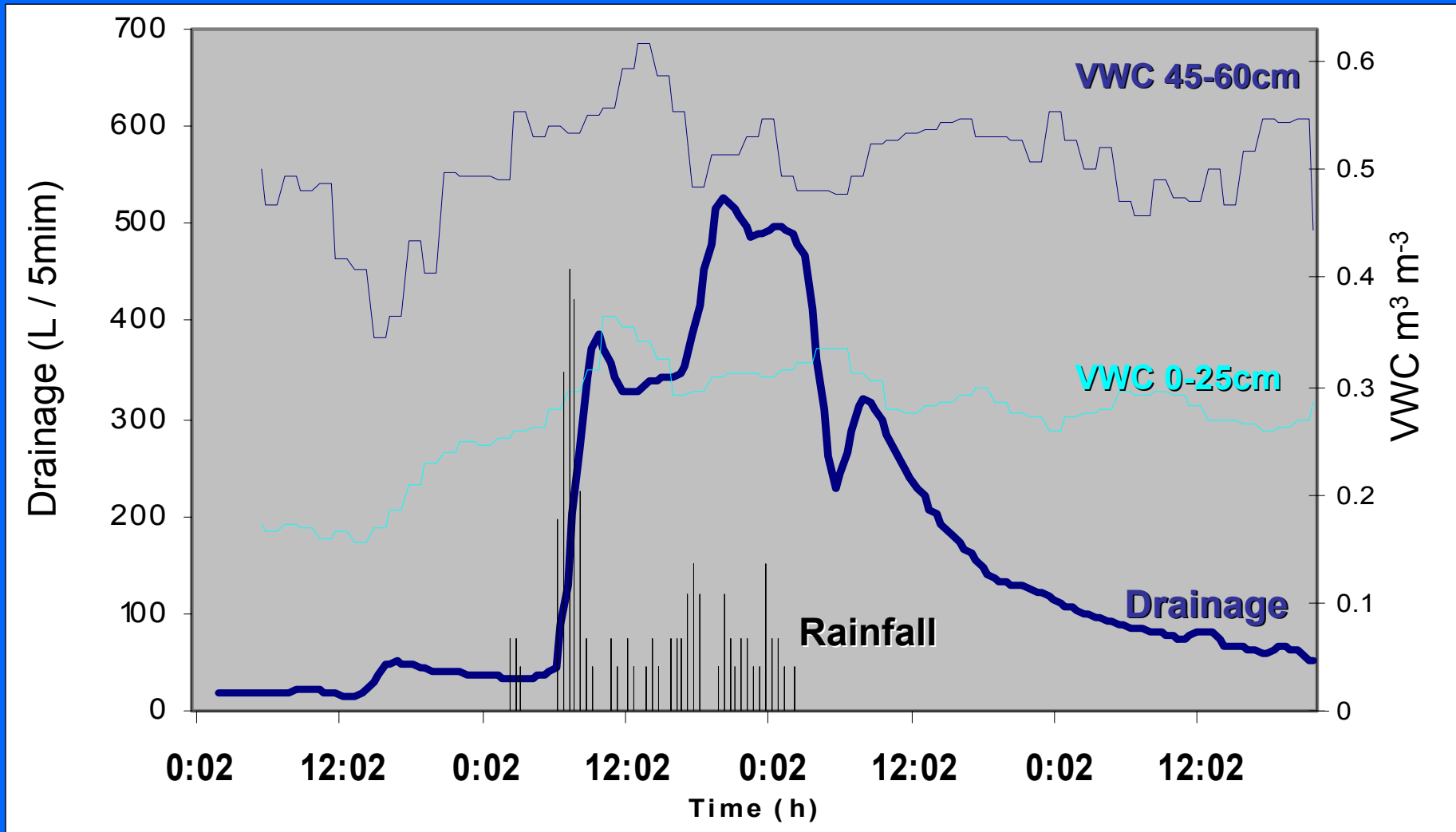
Monitoring program



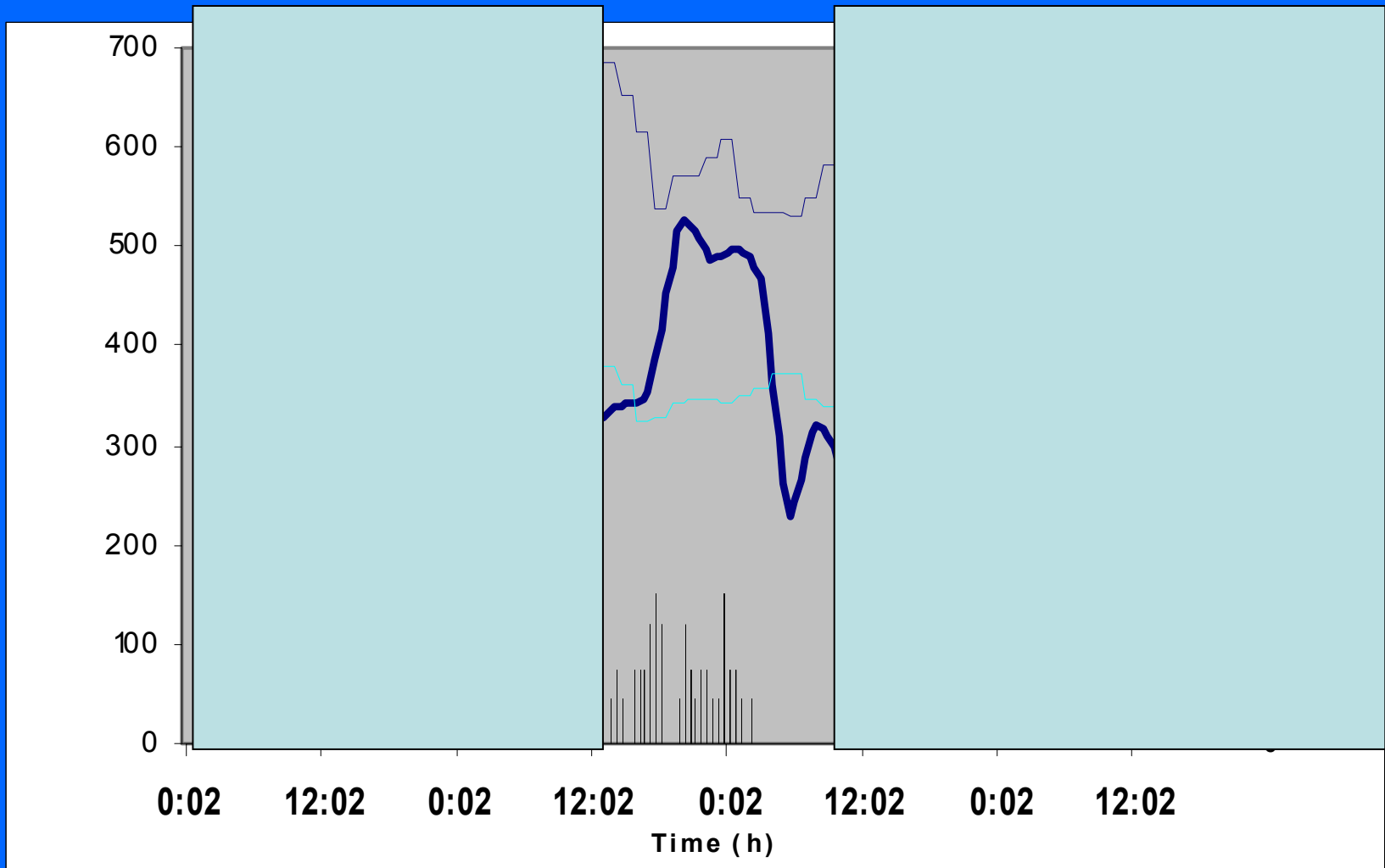
VWC = volumetric water content

Drainage patterns

Non-continuous event sequence: rainfall – soil water storage – drainage
24 Hrs. delay btw. rainfall and drainage



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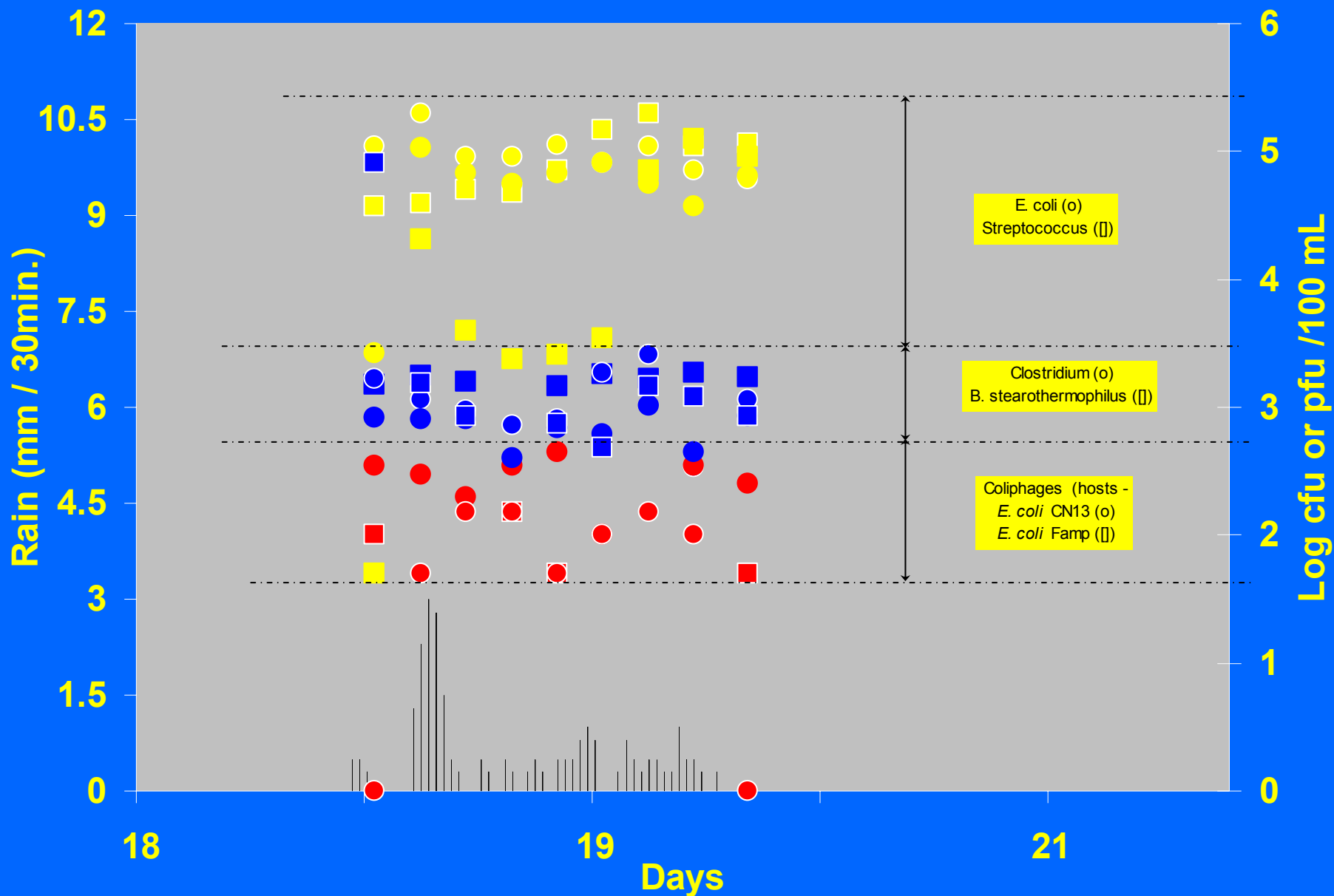
- **Pulse drainage – two stages:**
 - 1) **Accumulations of water at the bottom of the soil profile**
 - 2) **Pulse drainage**

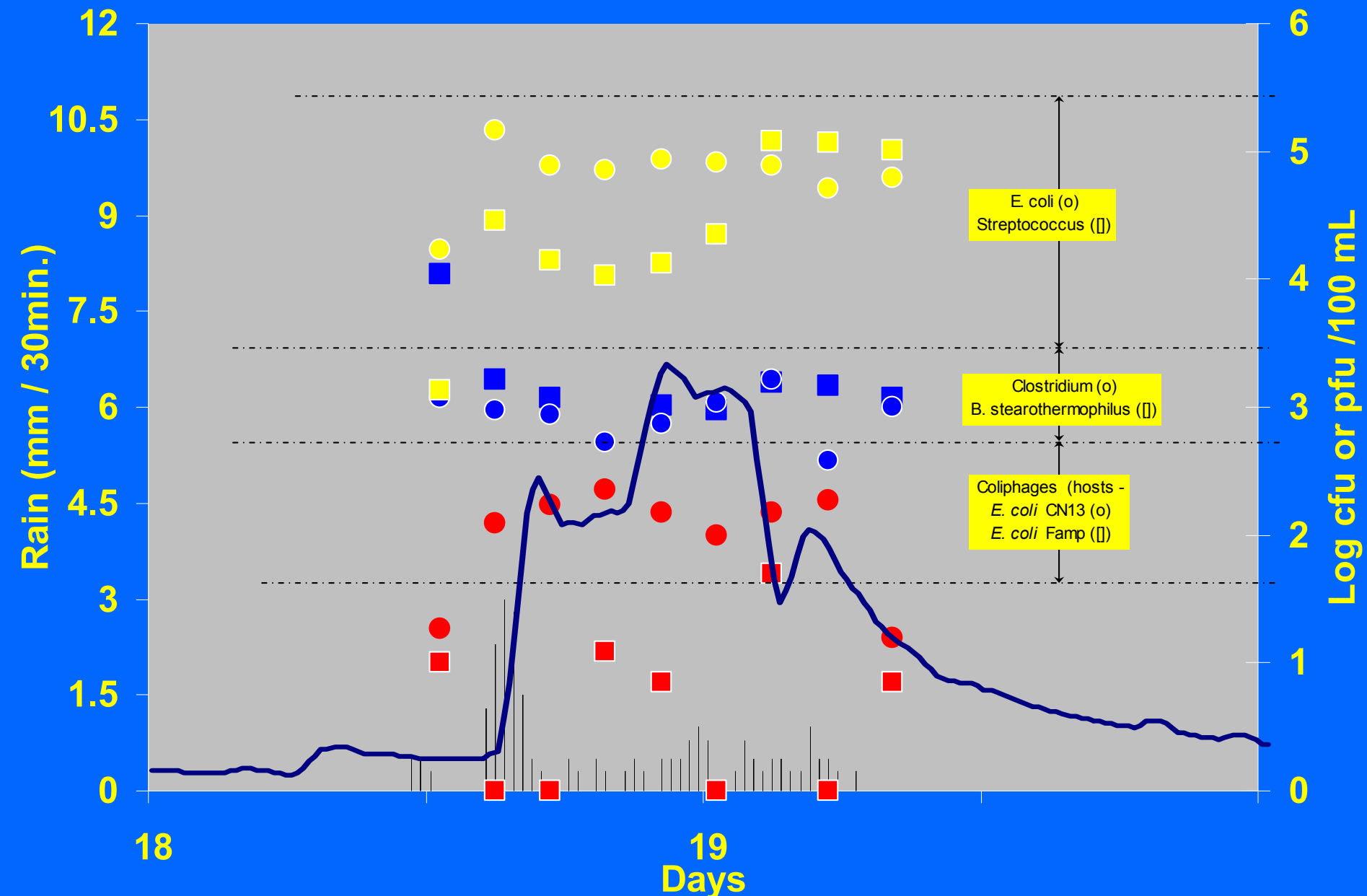
- **Possible implications:**
 - **Enhance diffusion = enhanced tile transport of viruses, nitrate**
 - » **To be verified by comparing with nitrate conc. of drainage**
 - **Short term - increased bacterial retention**
 - **Long term – enhanced re-suspension of persistent bacteria**

Microbial density in drainage

plots 5 and 6

2 days after the fall land application of liquid biosolids





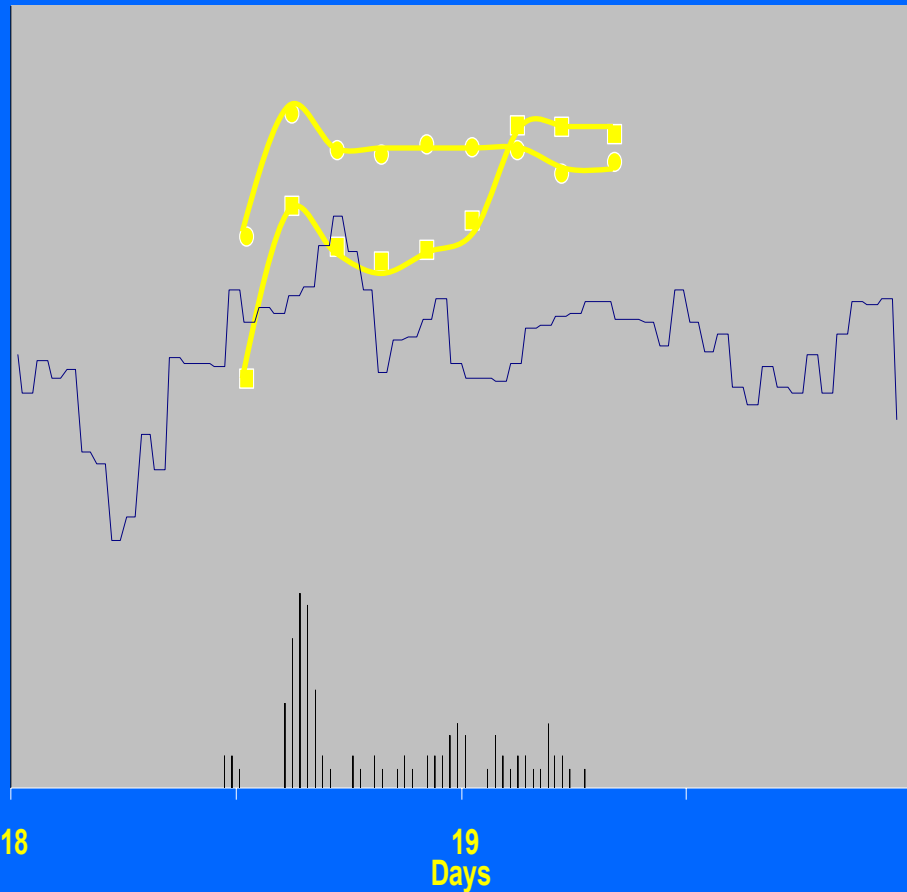
Presence of microbial indicators in drainage

- *E. coli* and *Streptococcus* present in largest numbers in drainage
- *Clostridium* also consistently present
- Phages transported at detectable levels mainly at increased drainage (low flow = enhanced phage retention)

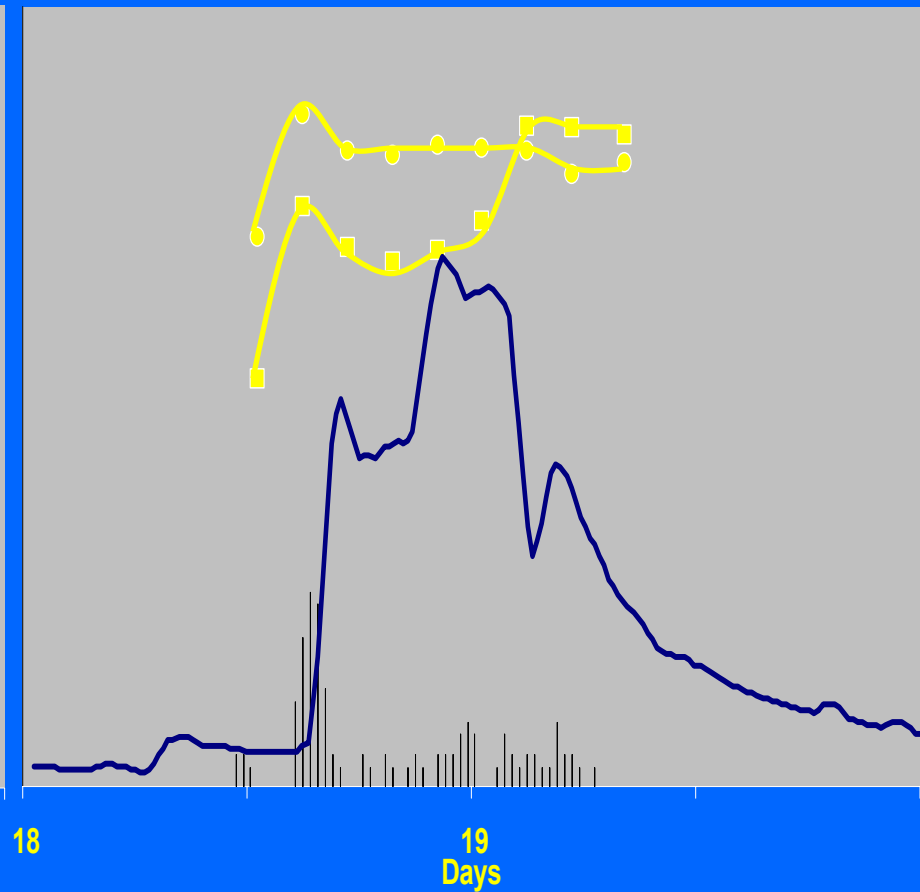
Variation of microbial density during the drainage event

E. coli (o) & *Streptococcus* (□)
- vegetative cells -

vs. VWC in the 45-60cm horizon



vs. tile drainage

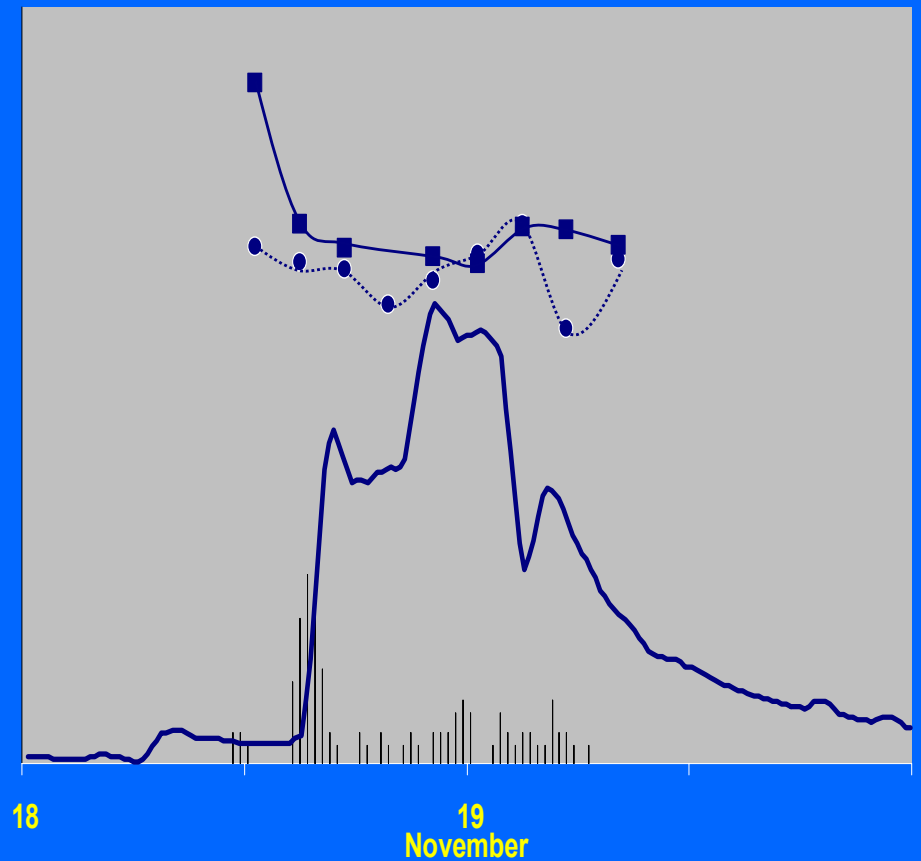
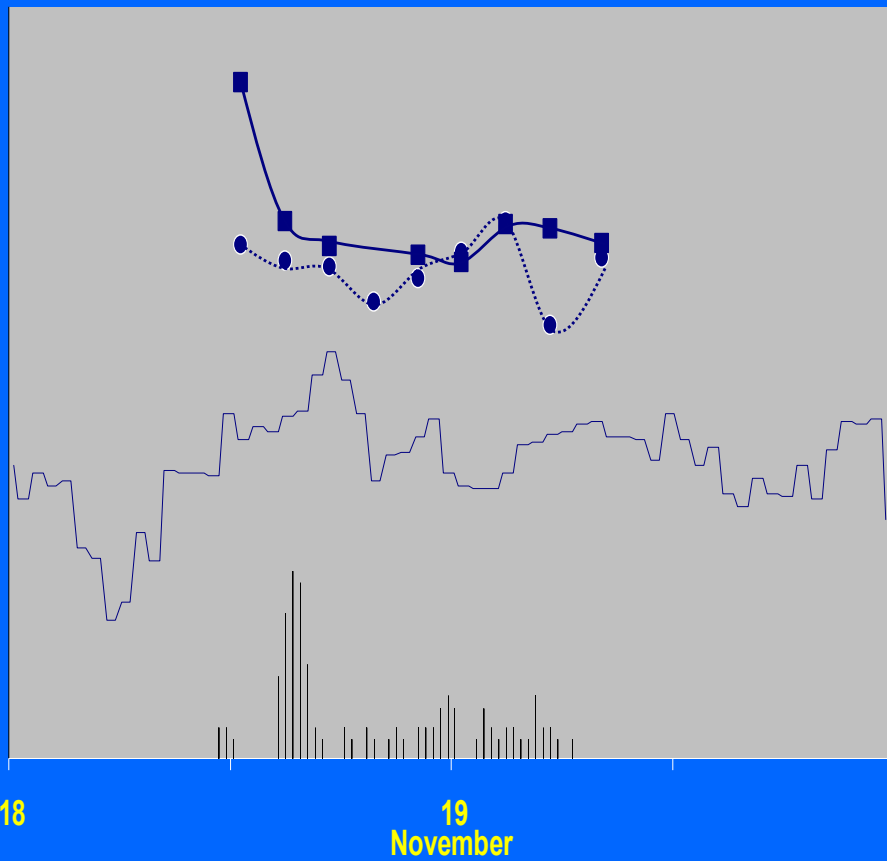


Variation of microbial density during the drainage event

Clostridium (o) & *B. stearothermophilus* (□)
- spores -

vs. VWC in the 45-60cm horizon

vs. tile drainage



Variation of microbial density during the drainage event

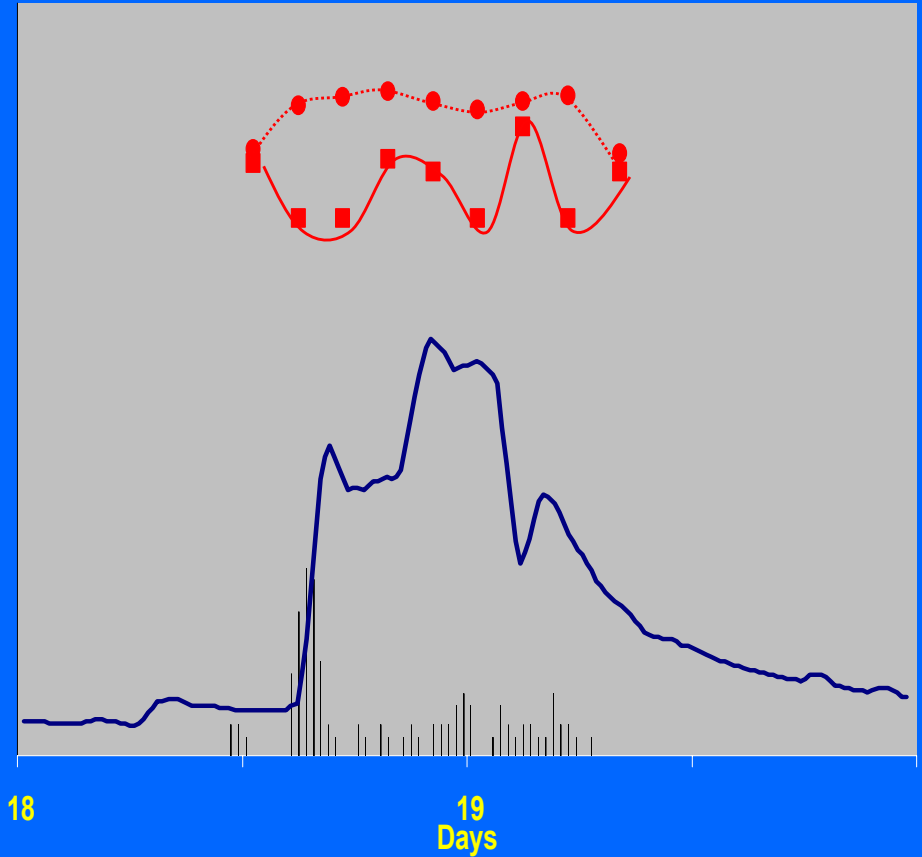
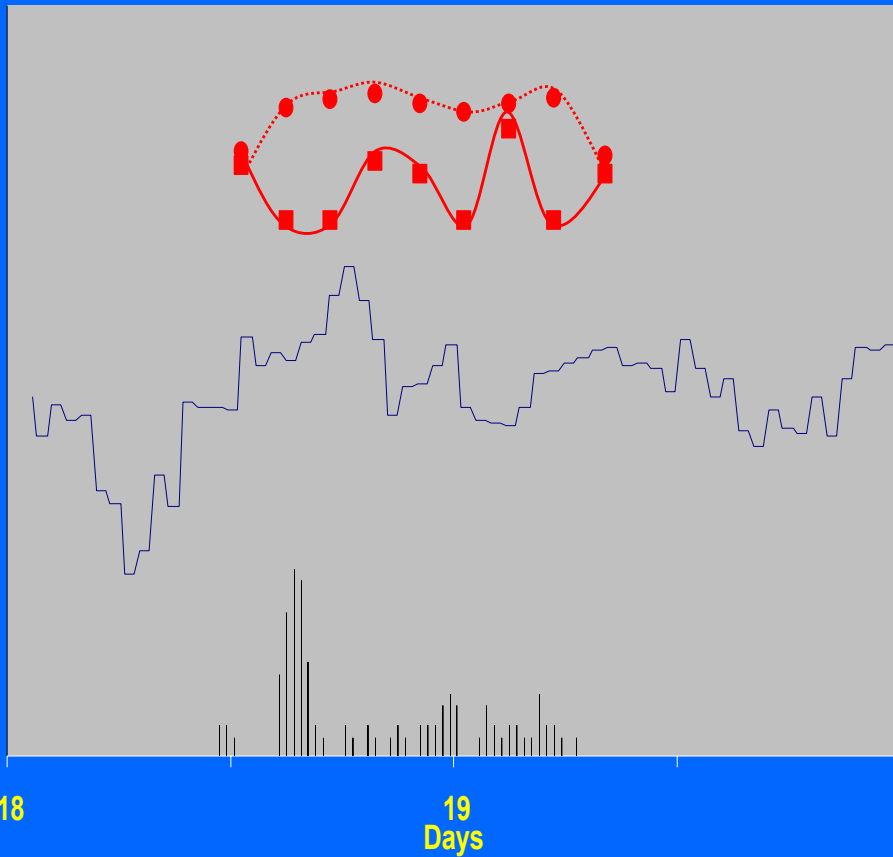
Coliphages

E. coli CN13 (o) & *E. coli* Famp (□)

- viral particles -

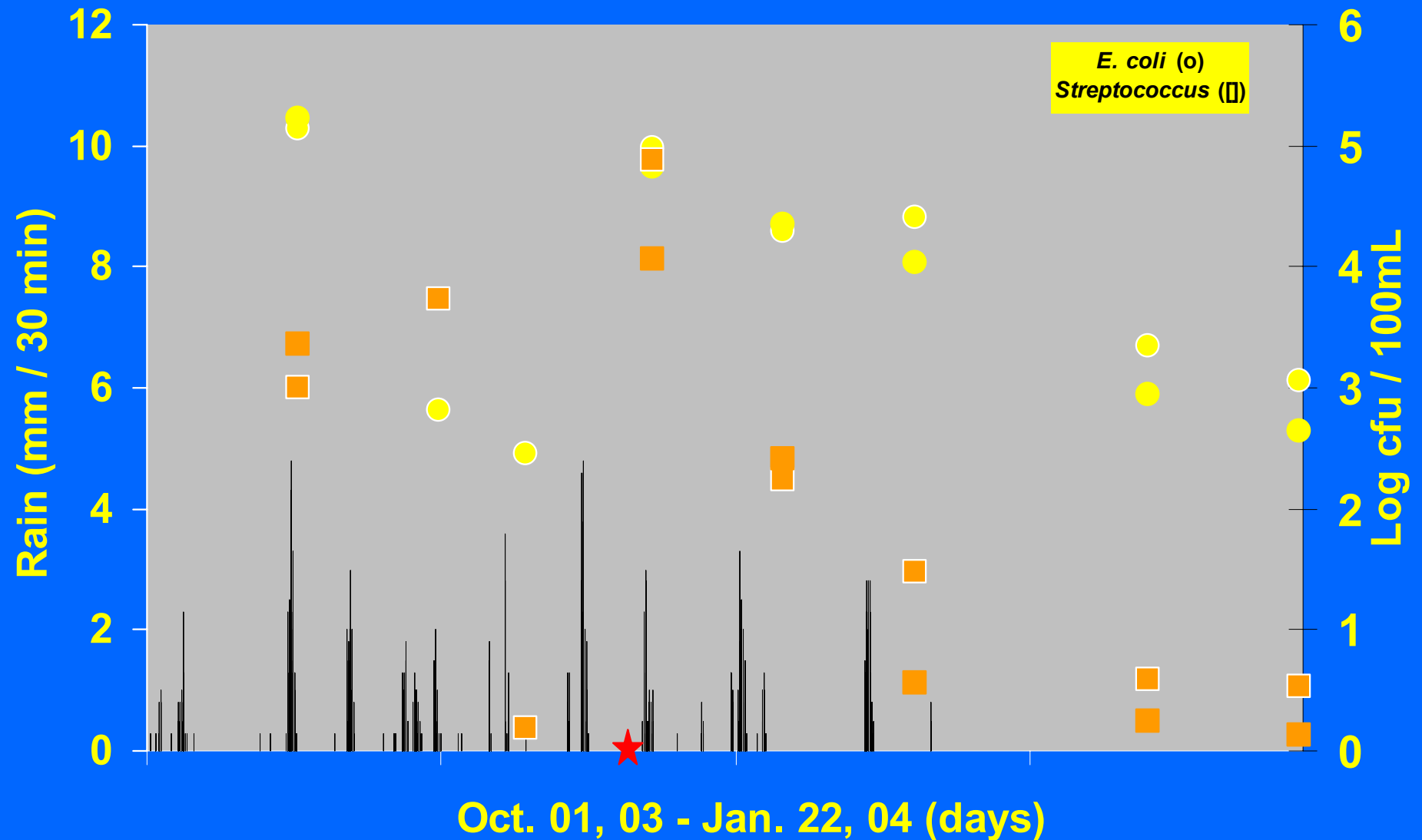
vs. VWC in the 45-60cm horizon

vs. tile drainage

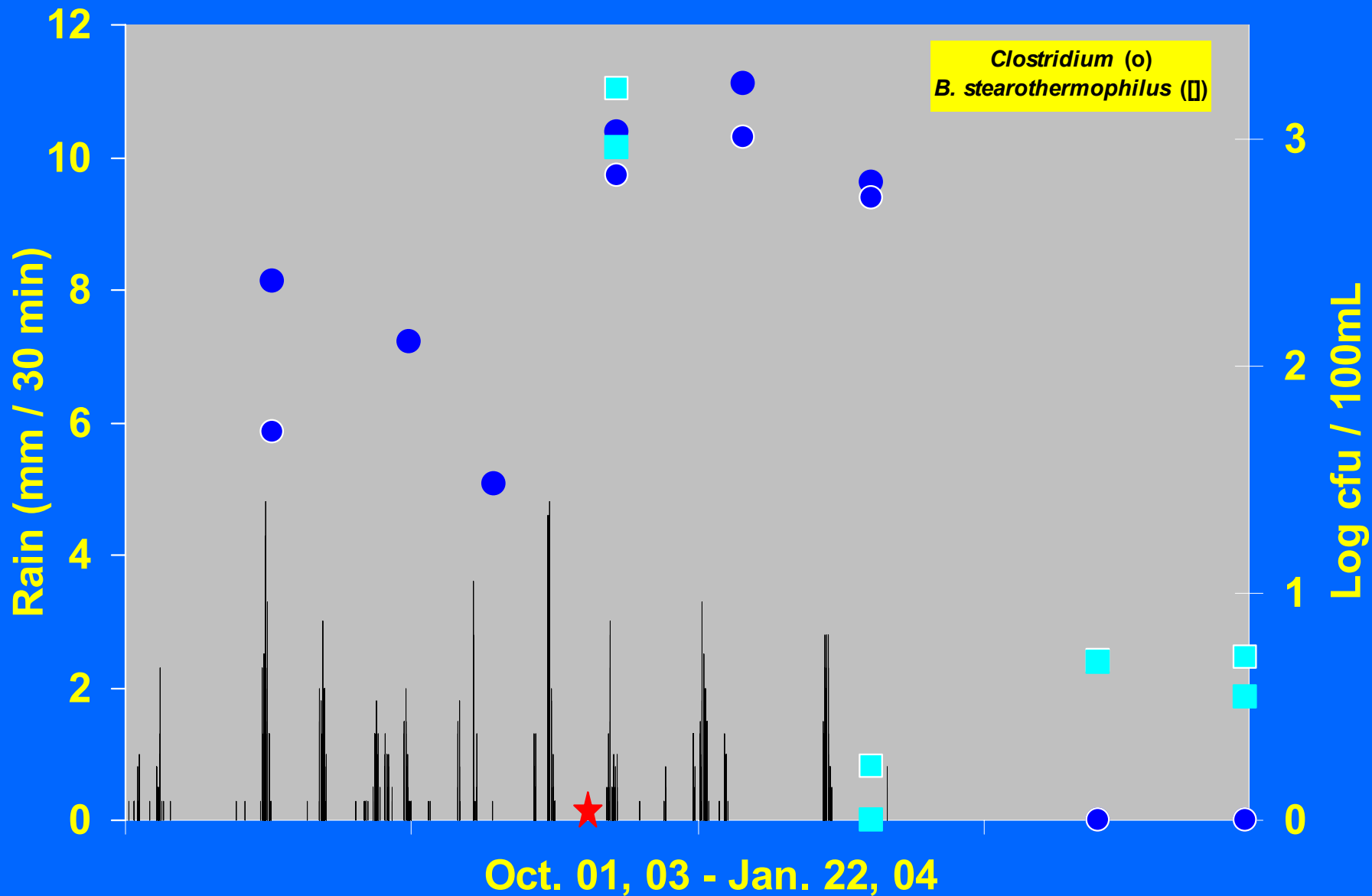


- **Vegetative cells – increased transport during drainage event**
- **Spores – decreased density during drainage event**
- **Viruses – increased density during drainage event**
- **For all – constant density during drainage peak(s); possibly due to the pulse drainage effect**

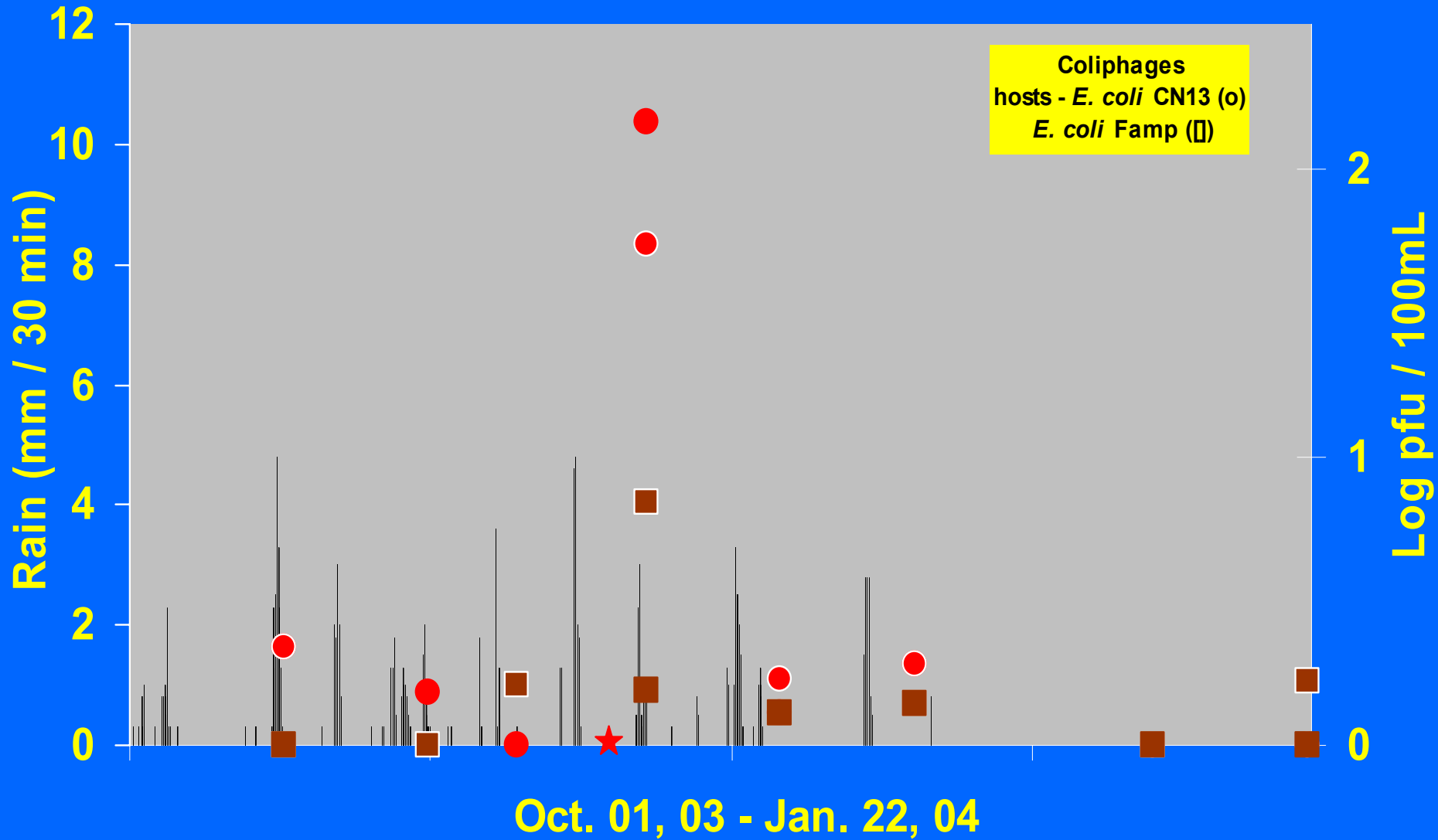
Microbial density in drainage –long term trends Before and after biosolids application



Microbial density in drainage –long term trends Before and after biosolids application



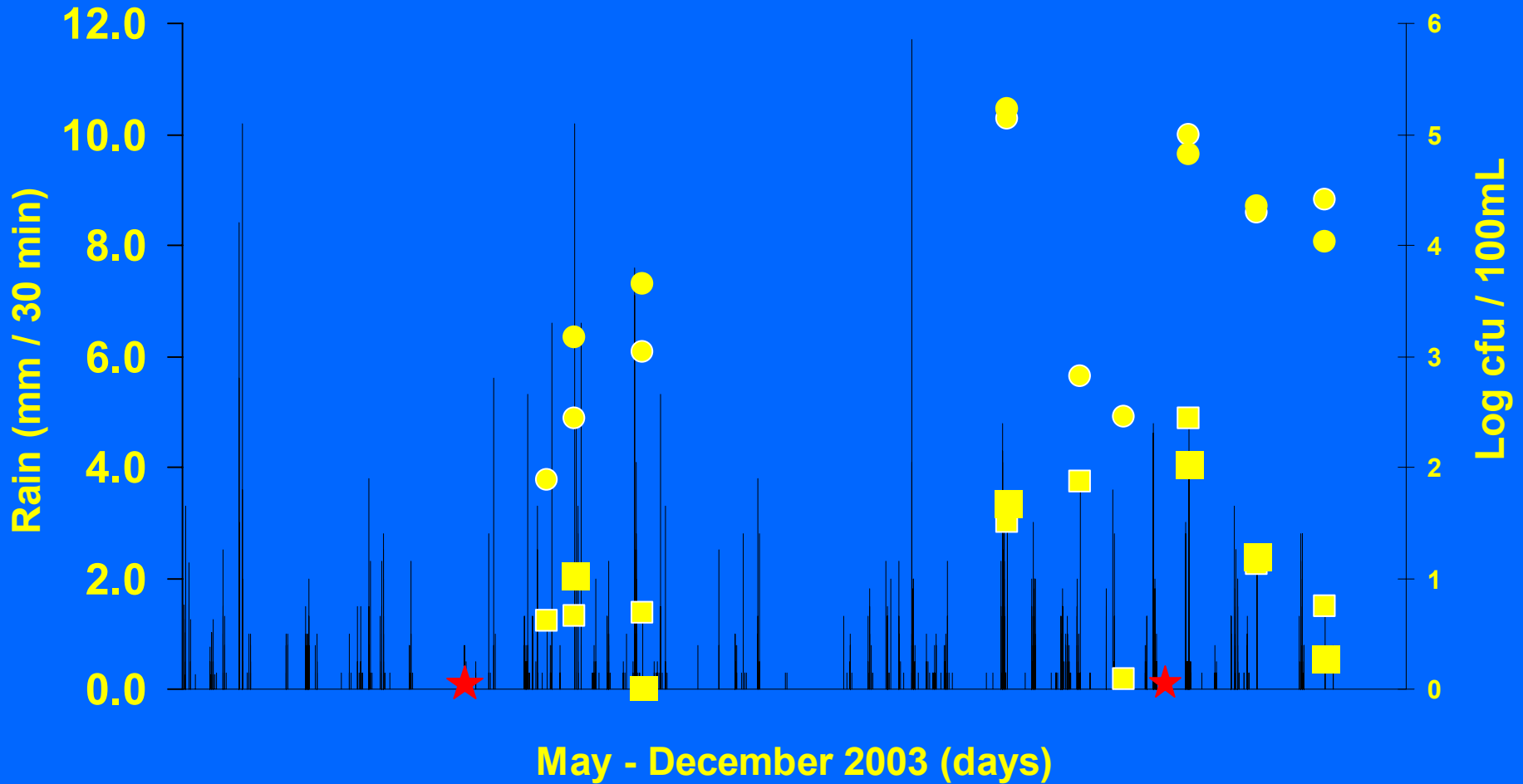
Microbial density in drainage –long term trends Before and after biosolids application



- ***E. coli* & *Streptococcus* – no dilution observed**
 - **Possibly post application resuscitation of soil *E. coli* & *Streptococcus***
- ***Clostridium*, *B. stearothermophilus* and coliphages – variable degrees of dilution**
 - **Land application as main source**

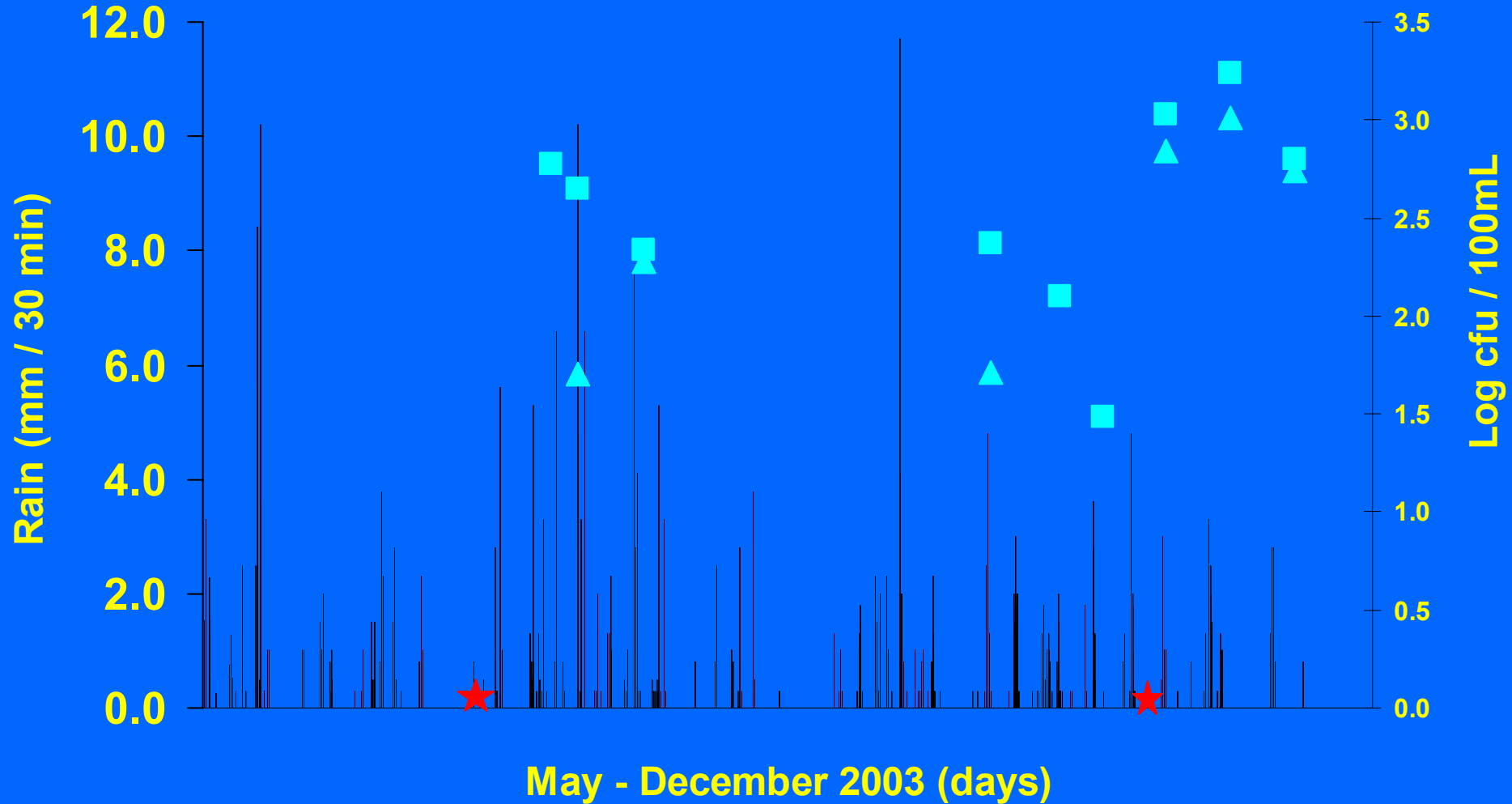
Microbial density in drainage following the summer and fall application of biosolids Plots 5 and 6

E. coli (o) & *Streptococcus* (□)



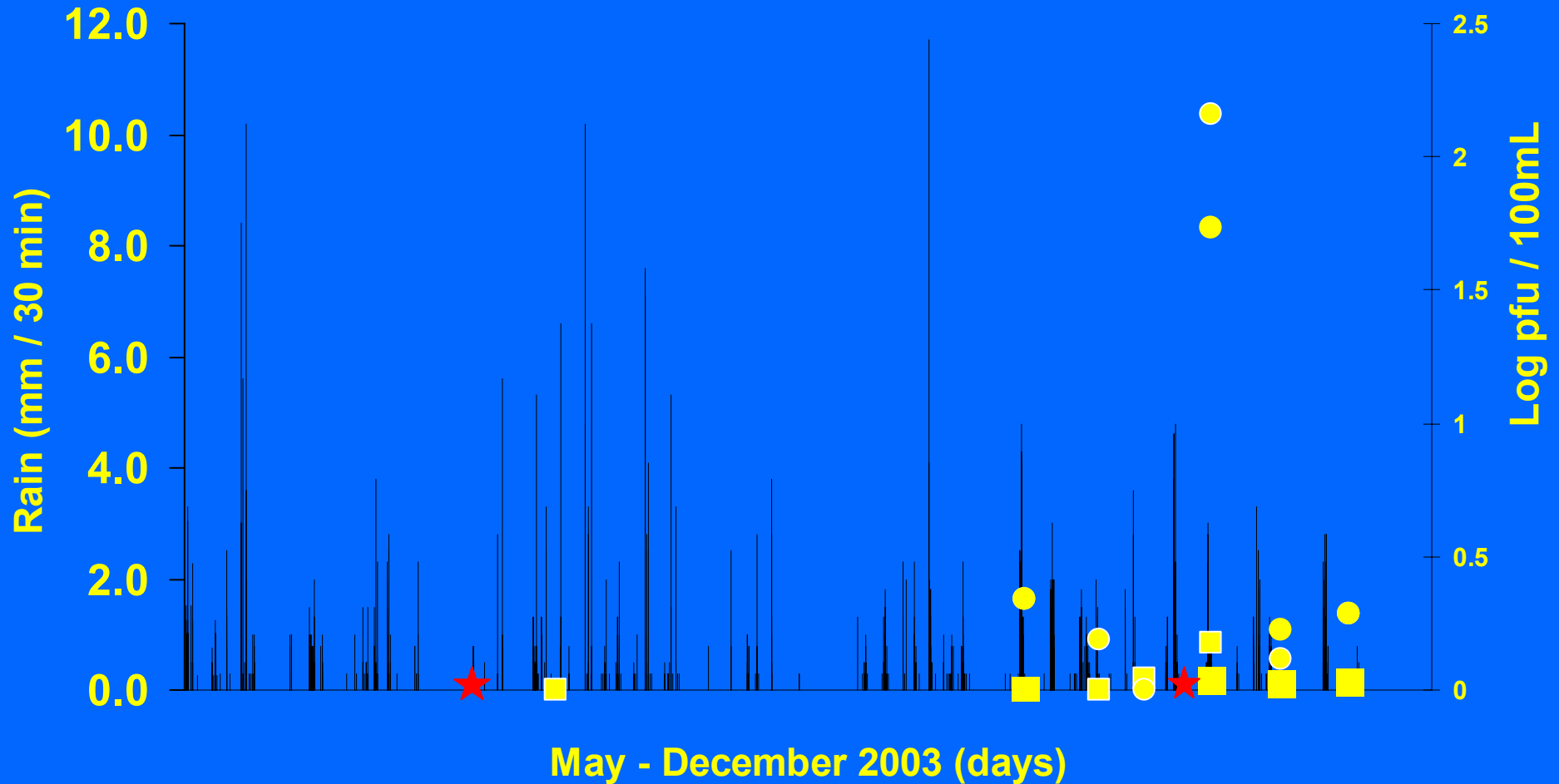
Microbial density in drainage following the summer and fall application of biosolids Plots 5 and 6

Clostridium



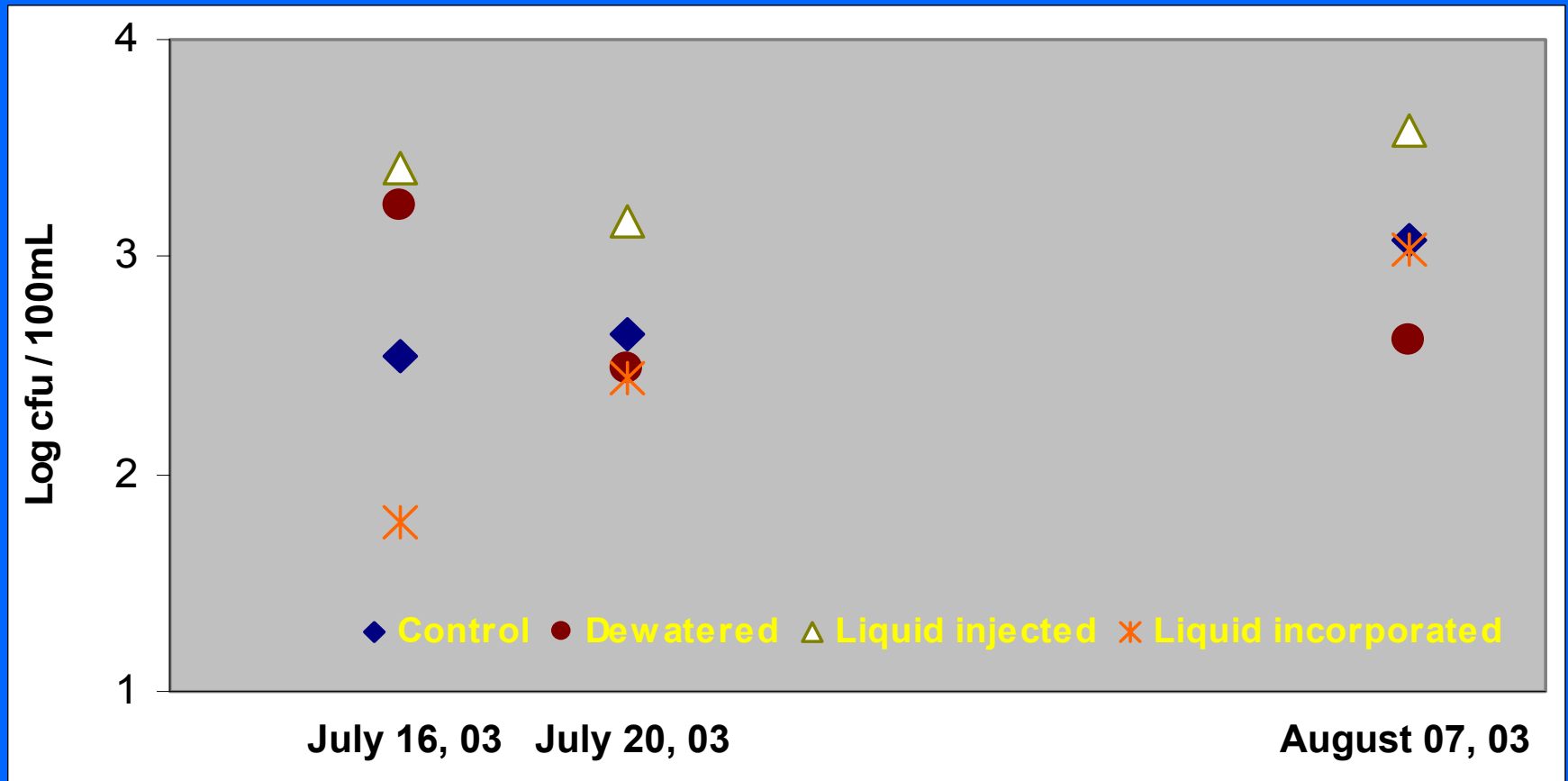
Microbial density in drainage following the summer and fall application of biosolids Plots 5 and 6

Coliphages



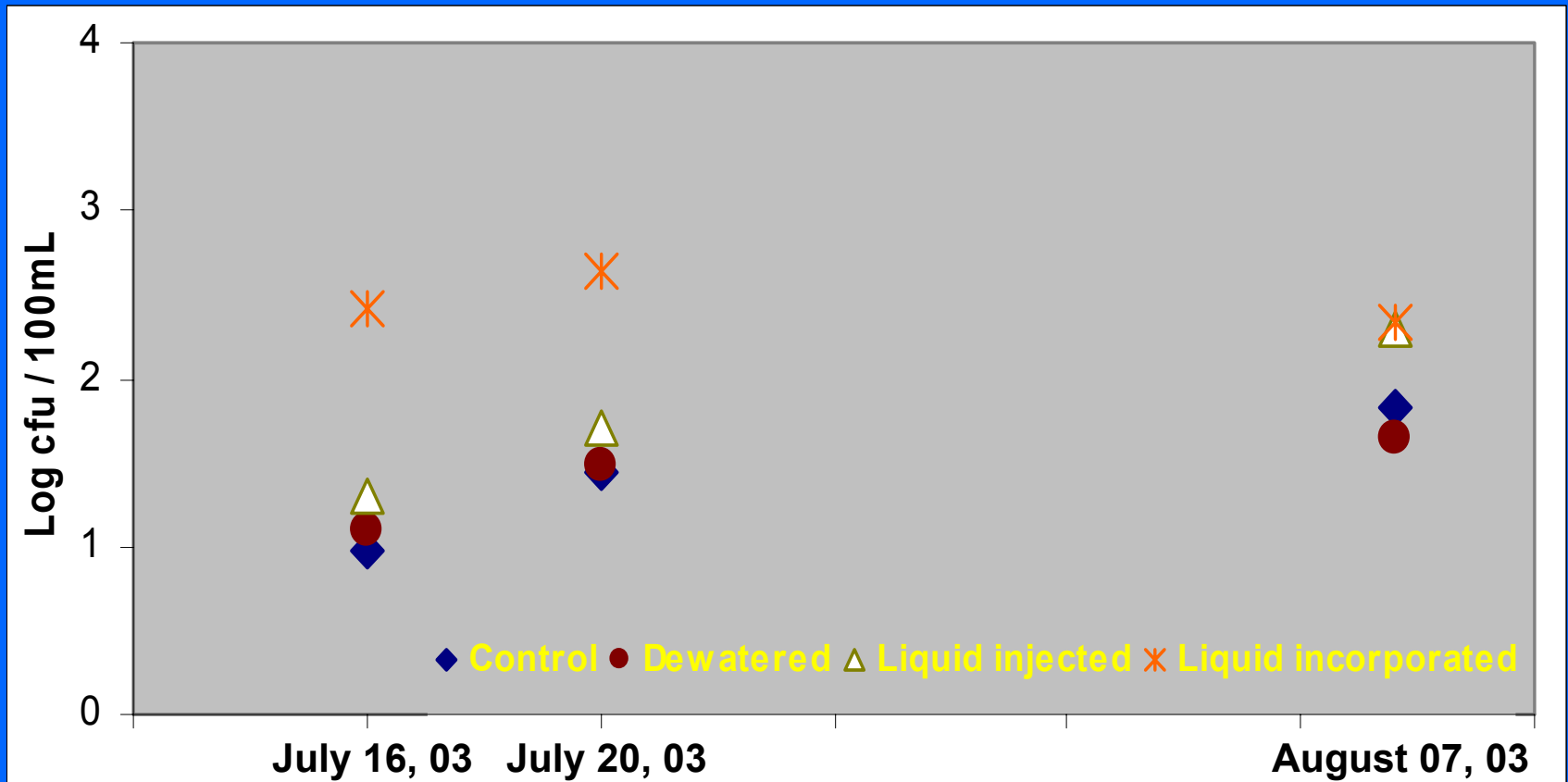
Microbial density in drainage following summer application of biosolids

E. coli



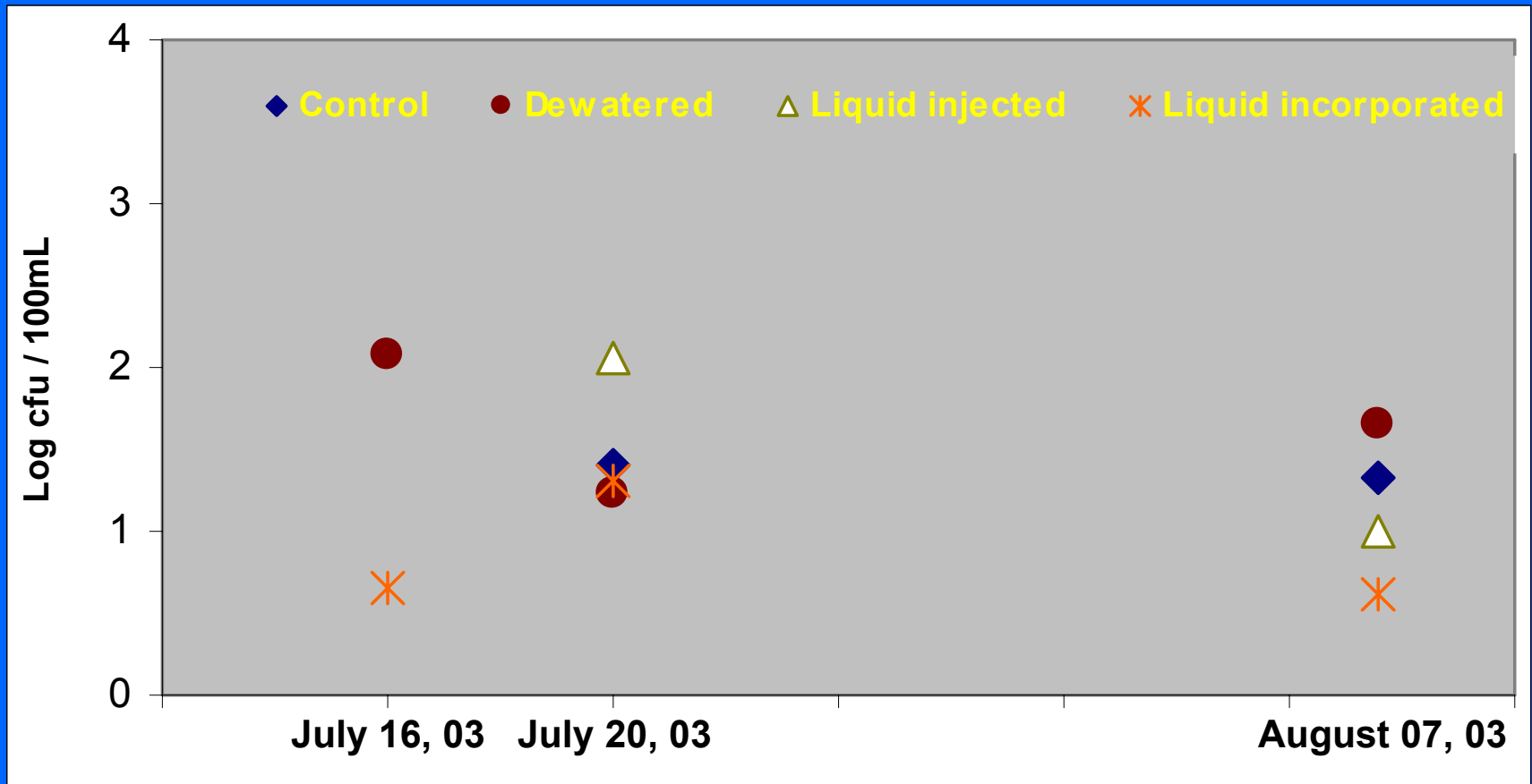
Microbial density in drainage following summer application of biosolids

Clostridium



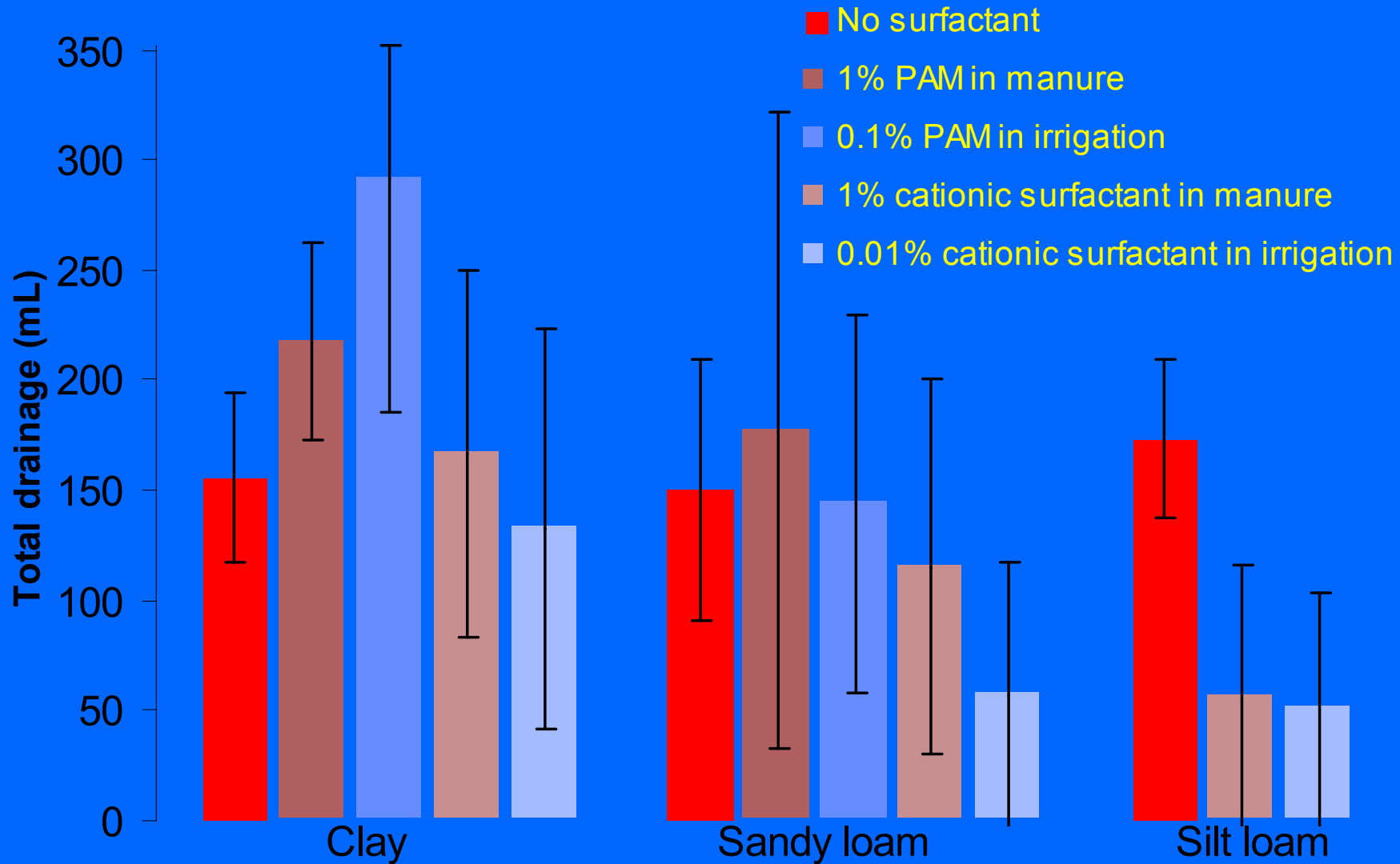
Microbial density in drainage following summer application of biosolids

Streptococcus



Significance of surfactants on microbial transport through soils

Drainage volumes after use of surfactant



Impact of surfactant use on the number of organisms present in the total drainage and their concentration in drainage as compared to manure only treatments

| FACTORS | | | ORGANISMS | | | | | | |
|------------|-----------------|-------------------------------|-----------------------------|-----------------------|--------------------------|-------------------------------|--|----------------------------|------------------------|
| Soil type | Surfactant type | Surfactant application method | <i>E. coli</i> | <i>Salmonella sp.</i> | <i>Streptococcus sp.</i> | <i>B. stearo-thermophilus</i> | <i>Clostridium sp.</i> | <i>phiX174 (coliphage)</i> | <i>MS2 (coliphage)</i> |
| | | | Total organisms in drainage | | | | Concentration of organisms in drainage | | |
| Clay | Non-ionic | To manure | = ↓ | = = | na | = = | na | na | na |
| | | To irrigation water | = = | = = | na | = = | na | na | na |
| | Cationic | To manure | = ↓ | ↓ ↓ | ↓ ↓ | = = | ↓ ↓ | = = | = = |
| | | To irrigation water | ↓ ↓ | ↓ ↓ | ↓ ↓ | ↓ ↓ | ↓ ↓ | = = | = = |
| Silt loam | Non-ionic | To manure | Na | na | Na | na | na | na | na |
| | | To irrigation water | Na | na | Na | Na | na | na | na |
| | Cationic | To manure | ↓ ↓ | = = | ↓ ↓ | ↓ ↓ | = = | = = | = = |
| | | To irrigation water | ↓ ↓ | = = | = = | = = | = = | = = | = = |
| Sandy loam | Non-ionic | To manure | ↑ = | ↑ = | na | = = | na | na | na |
| | | To irrigation water | ↑ = | ↑ = | na | = = | na | na | Na |
| | Cationic | To manure | = = | = = | ↑ = | = = | = = | = = | ↑ ↑ |
| | | To irrigation water | = ↓ | = = | = = | = = | = = | ↑ ↑ | = = |

Legend:

Total drainage – Amount of each microorganisms in total drainage (analysed as logcfu or logpfu) ; **Concentration** – Concentration of each microorganism in drainage (analysed as log cfu mL⁻¹ or log pfu mL⁻¹)

= No significant change in the number of the organisms as compared to the manure only treatment

↓ Significantly less organisms in the drainage as compared to the manure only treatment

↑ Significantly more organisms in the drainage as compared to the manure only treatment

na – no test performed

Impact of surfactants

- **The amount of bacteria transported was a function of their concentration in drainage water and the total amount of drainage both of which were independently affected by the surfactant treatments.**
- **Cationic surfactant added to irrigation water was generally more effective at reducing microbial transport through the soil columns; cationic surfactant added to the material (similar to dewatered biosolids) was less effective**

Conclusions

- **Low rate application did not result in obvious increase in microbial indicators monitored**
- **Subsequent high rate application of biosolids lead to a temporary increase in the microbial density in drainage (due to addition or resuscitation)**
- **Standard indicators of contamination – questionable**

- Further data collection will be performed to clarify significance of soil water content at time of application (time to ponding vs. drainage), application rate, management practices
- Continuing assessment of suitability of the microbial indicators of contamination
 - Source discrimination –
 - B. fragilis* HP40 phages – human source
 - B. fragilis* RYC 2056 phages – non-specific source
 - Spring 2004 tests will consider increased rates of application and will use the presence/absence test of *B. fragilis* phages as indicator for persistence of contaminant source. (PCR-DGGE & 16S rRNA sequencing confirmed *B. fragilis* as a component of biosolids)
- Lysimeter tests – drainage patterns as function of soil properties
 - tests in progress
- Significance of surfactants on microbial transport through soils
 - Tests to be finalised

