Determinants of Blackwaters in the South
Western Cape

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Abstract

Blackwater rivers and lakelets are a common feature of the landscape in the South Western Cape. Contrastingly, white rivers can also be found in the region. Key to the colour of blackwaters is the increased presence of dissolved organic carbon. The vegetation of the regions is known to possess large amounts of polyphenols and potentially low microbial decomposition. Therefore are the plant-soil dynamics a possible answer to variation in river colour? To answer this, the chemical correlates of rivers in the region, along with the in vitro leaching of the fynbos vegetation compared to other types and the effects of nutrient fertilisation on microbial activity in soil were determined. The results revealed that organic carbon, Fe and pH are significantly correlated with blackwaters. The Fe in the water appears to be bound to humic compounds. Fynbos vegetation is able to produce greater concentrations of polyphenol leachates (315 mg/l humic acid) compared to savanna species (246 mg/ humic acid) over a four day period. P fertilisations increased the concentrations of humic acids from 30 mg/l to 200 mg/l in some soils through their affinity to bind with humic compounds. The N fertilisations moderately increased the humic acid concentrations and in some cases lowered the concentration by 10 mg/l, indicating that it provided a nutrient source to the microbes for carbon breakdown. The interactions between humic compounds, nutrients and Fe are key to the formation of blackwaters in the region. Attached to this is the limited decomposition that takes place due to nutrient limitations. Therefore the interactions between the carbon leached from the vegetation and below ground activities are determinants of water colour in the South Western Cape.

Key-words: blackwaters; fynbos; humic compounds; dissolved organic carbon; plant leachates; microbial activity.
Introduction

Rivers in the South Western Cape are characteristically dark brown in colour, otherwise known as "blackwaters". Throughout the world these blackwaters tend to be associated with nutrient poor, sandy, podsolised soils, with the presence of evergreen sclerophyllous vegetation (Janzen, 1974). The fynbos biome, which occupies a large portion of the South Western Cape, is one such area (Cowling and Holmes, 1992). However some exceptions to the "blackwater" association exist as both clear and dark rivers can be found in the biome while in other parts of the country blackwaters are not common. The question then arises as to why there is this obvious difference in colouration between waters and what the environmental characteristics are, which make blackwaters a feature in the South Western Cape and the fynbos biome?

The dominant chemical characteristic associated with dark colouration of blackwaters is, the presence of dissolved organic carbon (DOC) which is consistent throughout blackwaters (Oliver, 1983; Gardiner, 1988, Midgley and Schafer, 1992; Sasche et al., 2005). DOC consists of various components such as carbohydrates, peptides, amino acids, pigments, phenols, tannins, humic and fulvic acids (Gardiner, 1988; Midgley and Schafer, 1992; Drever and Vance, 1994; Sasche et al., 2005). Yolk et al., (1997) found that of the various components of DOC, humic compounds accounted for 75 % of the DOC in the stream of an agricultural watershed. Similar quantities of humic compounds have also been noted at the head of estuaries (Otero et al., 2003) and in peat influenced surface waters of the temperate regions (Sasche et al., 2005). The origin of the majority of these humic compounds is the terrestrial environment.

The high concentrations of humic compounds in the fynbos vegetation are potentially a result of high phenolic contents found in many species (Cowling and Holmes, 1992; Midgley and Schafer, 1992). Phenolics are a common characteristic found in sclerophyllous vegetation (Janzen, 1974). Humic compounds, of which polyphenols (e.g. humic acid, tannic acid, phenol) are a constituent enter the soil column through two
pathways: (1) as leachates from living above and below ground plant organs and, (2) from above and below ground litter (Hattenschwiller and Vitousek, 2000). The above ground leachates are leached from the canopy in rain events and the below ground root exudates used in potential nutrient acquisition. McClaugherty, (1983) found for the sugar maple (Acer saccharum), the soluble polyphenols leached from the canopy (23 kg ha\(^{-1}\) yr\(^{-1}\)) were substantially less than that leached from the decomposing leaf litter (196 kg ha\(^{-1}\) yr\(^{-1}\)). Therefore it can be assumed that the litterfall provides a large source of polyphenol release into the soil. Studies on the annual litterfall rates in fynbos species indicate between 357 – 429 g m\(^{-2}\) for Leucospermum parile and 200 – 450 g m\(^{-2}\) for Protea repens (Mitchell et al., 1986; Mitchell and Coley, 1987). Compared to a tropical rainforest with rates ranging from 728 g m\(^{-2}\) - 1053 g m\(^{-2}\) annually (Spain, 1984), these rates can be considered to be low. However, the high levels of polyphenols in the litter are of greater importance than the amount of litter as evergreen sclerophyllous species which occur on oligotrophic soils (Janzen, 1974) such as in the fynbos should contain high concentrations. Glyphis and Puttlick (1988) recorded a mean phenol content of 7.55 % (w/w) in 23 species in the Cape Strandveld community; these levels are similar to those found in tropical rainforests (McKey et al., 1978). A study on the in vitro leaching of two sclerophyllous fynbos species indicated that riparian species do contain the polyphenols associated with blackwaters (Raubenheimer and Day, 1991). Fynbos species could therefore supply the polyphenols into the soil and contribute to the total amount of humic compounds found in the soils.

Below ground plant organs provide the second source to the accumulation of humic compounds in the soil column. The presence of these compounds is in part a consequence of root exudation. These exudates are released in the form of carboxylates, phenolics and mucilage (Lambers et al., 2006). The root exudates are considered to be an important component of nutrient acquisition, particularly in phosphorous deficient environments where P is often complexed with metals, to form Ca-, Fe- and A- phosphates (Keerthesinghe et al., 1998). Exudates are able to mobilize the scarce inorganic and organic P by complexing with metal cations through lignand exchange (Dakora and
Phillips, 2002; Lambers et al., 2003; Jones et al., 2003). Juszczuk et al., (2004) found in an 18 day study with the green bean (Phaseolus vulgaris L.) that plants exposed to a P deficient culture released five times more phenolics than those with sufficient P. Relevance to this and root exudates in the South Western Cape lies within their nutrient acquisition capabilities, in oligotrophic environments. The high usage of the exudates in such environments is supported by the presence of specific root adaptations known as cluster roots. The formation of these roots is induced through P deficiency (Lambers et al., 2006), they are able to increase root surface area, exudation and nutrient uptake. These roots are commonly found in many fynbos species (Lamont, 1983; Shane et al. 2004).

The potential fate of humic compounds in the soil lies within four pathways: (1) they may be degraded and mineralized by soil micro-organisms; (2) they can be transformed into insoluble and recalcitrant humic substances by polymerization and condensation reactions; (3) they may be adsorbed to clay minerals or they may form organo-metal complexes with Al and Fe ions (Hattenschwiler and Vitousek, 2000; Lambers et al., 2003). The organo-metal complexes created through this interaction or via the interaction of root exudates with metal phosphates can remain and build up in the profile to form ferricrete outcrops (Pate et al., 2001). These metal complexes can be found in fairly high concentrations in oligotrophic soils (Pate et al., 2001). However, in an oligotrophic system not all of these pathways may be possible. This is with a view to the potential microbial activity in the soil.

Humic compounds, polyphenols in particular are difficult for microbes to decompose and generally inhibit their activity in decomposition and nutrient cycling (Neuhauser and Hartenstein, 1978; Schimel et al., 1996). Combined with this is inability is the nutrient demand of micro-organisms which require an adequate supply of N and P so as to reproduce (Alexander, 1977; Cleveland et al., 2002). The oligotrophic soils of the South Western Cape posses low levels of N and P in particular (Witkowski and Mitchell, 1987;
Cowling and Holmes, 1997). This nutrient limitation on microbial proliferation is accentuated by the high retention rates of N (41%) and P (25-40%) by plants during leaf abscission (Mitchell et al, 1986), which affects nutrient cycling. In addition to this, polyphenols are able to alter N availability by complexing with proteins (Hattenschwiler and Vitousek, 2000). This situation would therefore appear to limit the possibility of microbial proliferation having an affect on decomposition and nutrient cycling. This potentially low microbial activity would then allow for a greater accumulation of soil carbon.

In this study we determined the chemical correlates associated with blackwaters in the South Western Cape. We then compared the leaf polyphenolic leachates of three vegetation types, savanna, fynbos and the indigenous forest vegetation found in the fynbos biome. The affects of limited nutrients on microbial activity and their influence on the humic compounds in soil were determined through N and P fertilisations. Subsequently the levels of polyphenols in the soils were monitored over time.
Materials and Methods

Water Sampling

500 ml water samples were collected from rivers and streams in five tertiary catchment regions (Figure 1): G22A, G40B, G40D, G40G, G40H and G22F (Surface Water Resources of South Africa, volume IV, 1990), in the quaternary catchment region of the Greater Berg River in South Western Cape, samples were frozen within 3 hours of collection for further analysis. The samples included a wide range of colouration, from completely clear to dark brown.

Fig 1: Map of the South Western Cape tertiary catchment regions, indicating the sampled catchments (after Midgley et al., 1994)
Correlates of water colour

The colour the river water samples was measured spectrophotometrically. Sample were analysed at three wavelengths, 230, 290 and 400 nm. The wavelength which illustrated the greatest variation across all samples was used in further analysis. Measurements were performed on a Power Wave XS plate reader (BIO-TEK® Intruments, Inc. Vermont, USA) relative to a distilled water blank.

River water was analysed for pH, electrical conductivity (EC), Na, K, Ca, Mg, Mn, Fe, B, Cu, Zn, P, Cl, CO₃^-, HCO₃^-, SO₄^-, NH₄^+ and NO₃^- according to the methods set out by Clesceri et al., (1998) for the examination of water and wastewater. An ICP- MS with suitable standards performed the final measurements (BemLab, De Beers Rd, Somerset West, South Africa).

The soluble polyphenolic concentrations were determined using the Folin-Ciocalteu phenol reagent. The method followed Box (1983), using the alkaline carbonate/tartrate as the supporting solution (Kloster, 1974; American Public Health Association, 1976). The method involved the addition of 40 μl of carbonate/tartrate (200 g of sodium carbonate and 12 g sodium tartrate dissolved in 750 ml of hot deionized water and diluted to 1 L) solution and 15 μl Folin-Ciocalteu phenol reagent (Merck, Damstadt, Germany) to 250 μl of filtered sample. The mixture was then left for 60 minutes and the absorbance’s were then measured at 700 nm using a Power Waver XS plate reader. A calibration curve for a humic acid (Sigma-Aldrick, Steinheim, Germany) standard (0 – 250 mg/l, r² = 0.9871) was prepared.

Plant Polyphenolic Leachates

Leaf material samples of twelve of species and a bunch grass sample in three different vegetation types, fynbos (Proteaceae: Leucospermum guenzii, Protea repens; Restionaceae: Chondropetalium tectorum, Thamnochortus insignis; Ericaceae: Erica
discolour, *Erica baccans*), Cape Floristic Region forest (*Olea capensis* and *Podocarpus elongates*) and savanna (*Bauhinia tomentosa*, *Erythrina lysistema*, *Acacia burkeii*, *Acacia sieberiana*). The bunch grass consisted of several species and was collected in the Hluhluwe-Umfolozi reserve, the other samples were collected in the Kirstenbosch Botanical Gardens. Each sample was dried at 40°C for four days prior to being milled to pass through a 0.5 mm mesh screen. A 0.2 g sample of each milled sample was then soaked in 40 ml of distilled water and incubated in dark room at 25°C. A sub sample of the resulting solution was taken from 0.25; 0.5; 1; 2; 4 days from the start. The soluble polyphenolic concentrations were analysed using the modified Folin-Ciocalteu method and expressed as mg humic acid l⁻¹(HA).

**Microbial Decomposition**

Five different Fynbos soils, collected from Koeelbaai, Elim, Molshoop, Tradouw and Stanford were selected for this analysis (See Table 1). All of the soils have a sandy texture apart from the Elim sample which is a limestone with a coarse aggregate. Portions of the soils were prepared and analysed for pH on both KCL and water extracts and electrical using the methods described in the Handbook of Standard Soil Testing Methods for Advisor Purposes (The Non-Affiliated Soil Analysis Work Committee, 1990). N and P were measured using ICP-MS analysis and a LECO-nitrogen analyser with suitable standards (BemLab).

To determine the affects and degree of microbial decomposition on humic acids in each soil two fertilisation sets were initiated. The first set of fertilisations, were applied with distilled water (Experiment 1), while the others were initiated with a 34 mg/l humic acid solution (Experiment 2). This concentration was found to be the average humic acid concentration of the collected river waters. The three fertilisation treatments applied were, 0.5 mM NH₄NO₃, 0.2 mM K₂HPO₄ and a combined 0.5 mM NH₄NO₃ and 0.2 mM K₂HPO₄ solution. For each treatment, with or without nutrient fertilisation, a 20 ml
solution was added to 10g of soil so as to saturate the soil. The samples were then incubated in a dark 25°C room. A sub sample of the resulting solution was taken 0; 1; 2; 5 and 10 days from the start. The soluble polyphenolic concentrations of the solutions were analysed using the modified Folin-Ciocalteu method and expressed as mg humic acid l⁻¹.

**Data Analysis**

Pearson coefficient of correlation was used to determine the strength and significance of regressions in all correlations used against the absorbance at the 230 nm, 290 and 400 nm wavelengths. The humic acid concentrations from the *in vitro* and fertilization experiments were log transformed. The significant differences between *in vitro* leaching of vegetation types were determined using one-way repeated measures ANOVA. Results of post-hoc multiple comparisons (Turkey’s LSD test) are reported at α = 0.05. All data were analysed using STATISTICA© v 7.0. (Statsoft, Inc. Oklahoma, USA)
Results

Correlates of water colour

In the colour analysis, absorbances taken at 230 nm showed the greatest variability across all samples i.e. showed the largest range in colouration. For this reason we decided to use the 230 nm absorbencies and proceeded to formulate all correlations at this wavelength.

The pH values of the sampled waters ranged between 4.2 and 7.1 with the majority of the samples being predominantly acidic. The correlation of between colour and pH (Figure 2) yielded a significant, negative correlation. Samples that had a lower absorbance had high pH values compared to those with higher absorbance's. Carbon, iron and humic acid concentration (Figure 2) produced significant, positive correlations with colour. The relationship between iron and humic acid concentration (Figure 3) yielded a significant and positive correlation. Other elements and compounds analysed did form significant relationships with the sampled waters absorbance's at 230 nm.

Plant Leachates

The sampled leaf materials all revealed a release of humic acid (Figure 4). The total concentrations released from all species ranged from 50 mg/l to 550 mg/l humic acid over the incubation period. The rates of release (Figure 5) between vegetation types was found to be significantly different, with the fynbos and CFR forest species producing the highest rates and the savanna species the lowest. Within the fynbos the Restionaceae species revealed very different release patterns. Chonropetalium tectorum rapidly released humic acids within the first day and reached its maximum concentration after two days. Subsequently the concentration began to decrease. Thamnochortus insignis produced a slow but gradual release; however its humic acid release was the lowest within the fynbos species. The rates of humic acid release (Figure 5) do not reflect as great differences, with Thamnochortus insignis producing a marginally higher rate than
Chonropetalium tectorum. The quantities of humic acids released by the Ericaceae species were also found to be different from each other but both with slow initial releases. Erica baccans only produced a quantifiable change in humic acid concentration after twelve hours, after this a gradual release took place. Erica discolor released a lower concentration over the same time period, in comparison. The differences in rates of release were small with Erica baccans producing the higher rate. The two Proteaceae species (Leucospermum guenzii and Protea repens) tracked each others release patterns. Both species released a large proportion of their humic acids within the first day; from there they reached equilibrium with Leucospermum guenzii having a greater total concentration. This similarity is reproduced in the rates of release and collectively produced the second highest release rate over the four days. Olea capensis and Podocarpus elongatus recorded the highest total concentrations amongst all of the species. Olea capensis released its greatest proportion of humic acid after one day and then reached equilibrium. Podocarpus elongatus maximum release was obtained after two days but produced a higher total concentration Olea capensis. However, the rate of release in Olea capensis was 50 mg/g/day greater than in Podocarpus elongatus.

In the savanna species, the legumes showed similar patterns of release, with the greatest amount of humic acid released in the first day of incubation. Bauhinia tomentosa released the highest concentration followed by Acacia burkeii and Acacia sieberianna. The non-legume tree species, Erythrina lysistema followed a similar pattern to the legumes by releasing close to all of its humic acids within the first day and then reaching equilibrium. The results from the bunch grass are in complete contrast to all of the other savanna samples. While most species released the majority of the humic acids within the first day, the bunch grass sample only began a consistent release after day one and recorded the lowest rate of release amongst all samples.
Microbial Decomposition

In experiment 1 all five soils (Figure 6) showed changes in humic acid concentrations. The four treatments produced two fairly clear cut groups in soil humic acid concentration, with the P and N+P treatment being similarly high and the N and distilled water similarly low, throughout all of the soils types. This difference and that between the N and distilled water were found to be significant. P fertilization linearly increased the humic acid concentration over the 10 day incubation period with all soils apart from the Molshoop sample, where the concentration decreased after two days from initiation and then reached a steady state. In the Koeelbaai soil, the P rapidly increased the humic acid concentration in the first five days and then proceeded to remain constant. The N+P treatment closely followed the pattern of the P, showing linear increases in all soils except in Molshoop sample. In the Molshoop soil the humic acid concentration rapidly increased in the first two days but declined afterwards. The effective increases that this treatment had on the humic acid concentrations were, however, less than with the P treatment. The N and distilled water treatments, the second visual group, produced increases in humic acid concentration by day two in all soils. Throughout the incubation period the distilled water produced higher concentrations than the N, with the Elim and Molshoop soils being the exception. Both treatments revealed decreases in humic acid in all soils apart from Molshoop soil where they continued to increase until day five. Post day five a decrease in humic acid occurred in the Koeelbaai, Elim and Molshoop soils. In the Tradouw soil, the distilled water treatment continued to increase past day five, while the N treatment began to decrease after day five. In the Stanford soil, both treatments marginally increased post day five.

The experimental treatments with the humic acid addition revealed similar patterns to those observed in experiment 1 (Figure 7). However each treatment was found to be significantly different from each other. The HA+P and HA+N+P treatments linearly increased the humic acid concentration in all soils, with both treatments closely linked.
The HA+N treatment increased the humic acids in within the Koeelbaai soil in the first day and then began to gradually decrease over the rest of the incubation. In the three other soils the same increased can was found after a day. The humic acid concentration then remained fairly constant with some small increases in the Elim and Stanford soils. The HA followed a similar pattern to the HA+N in Koeelbaai soil. The Elim, illustrated decrease in humic acid after five days. In all other soils the HA produced a gradual increased in humic acid.
Discussion

The analysis of several black and clearwater systems confirmed the effects of organic carbon on colouration and the pH levels (Figures 2). Dark waters contained higher levels of organic carbon and possessed a lower pH value. The correlation with pH is most likely a result of the increased concentrations of organic carbon, which will cause a decrease in water pH. The oligotrophic soils of the area are highly leached and possess a low pH; this level is very much influenced by plant exudates and leachates such as decaying polyphenols (Hättenschwiler and Vitousek, 2000). The correlations between absorbance and humic acid are similar to those of Gardiner, (1988) who also noted an increase in humic compounds with water colour. Therefore, not only does organic carbon influence water colour but more specifically this influence is a result of the accumulation of humic acids in this study, which are from a terrestrial source. The humic acids most certainly in turn influence the pH of the surrounding waters and indeed assist in the lowering it in blackwaters.

The correlation between Fe and humic acid indicates that they are probably bound. Jones and Brassington (1998) showed that anions such as organic acids and polyphenols readily bind to ferrihydrite and that this sorption was accentuated when the pH of the surrounding solution was lowered. Humic compounds have the ability to lower the pH of their surrounding environment which therefore creates a higher affinity for them to bind to metal ions (Jones and Brassington, 1998). Further evidence for this lies in the interactions between root exudates and metal phosphates. The ability of organic exudates to complex with metal phosphates to form organo-metal complexes (Dakora and Phillips, 2002; Lambers et al., 2003; Jones et al., 2003) will allow for a higher probability of Fe and humic compounds being associated with each other. In a biome where nutrient acquisition is a critical process (Lambers et al., 2006) and root exudation is higher through the formation of cluster roots, greater levels of organo-metal formations will more frequently occur. These complexes can then be leached out of the soil column.
during rainfall events or assist in the formation of ferricrete soils (Pate et al., 2001). In turn these complexes can potentially be the source of high levels of carbon and iron in blackwaters. The sandstone derived soils with a low number of binding sites, high porosity and low pH provide for such a process to take place. Increased ability for leaching could possibly provide support for the statement that the blackwater rivers in the area become darker during the winter months, the rainfall period.

Two previous studies have been carried out on the in vitro leaching of fynbos species (Raubenheimer and Day, 1991) and the indigenous forest species (Midgley and Schafer, 1992). In both studies fynbos and forest species leached polyphenols from leaf material and darkened the surrounding solution. The two riparian species used by Raubenheimer and Day, (1991) produced different levels of total polyphenol release in the incubation; however both species leachates lowered the pH considerably. A similar study has not been carried out on other species from vegetation types to compare whether vegetation of the fynbos biome produces more polyphenols leachates in its litter than in other areas. The results (Figure 5) from this study showed that the three vegetation types produced significantly different amounts of humic acid when compared to each other. The CFR forest species produced the highest followed by the fynbos and savanna species. Midgley and Schafer (1992) also found that the CFR forest species produced a greater darkening of water in comparison to fynbos species.

The marginal differences between the fynbos and forest species indicate that most indigenous species in the fynbos biome can be considered to have the capacity for large amounts of polyphenol leachates to be released from their litter compared to other vegetation types. It might be argued that they have the potential to contribute to the humic compounds pool in the soil column, but at the same time they don't have sufficient litterfall rates as compared to rainforest where blackwaters also are found. The rates of release (Figure 5) confound this as the fynbos and forest species were able to release greater amounts of humic acids compared to savanna species over the same period.
Therefore the litterfall rates in other vegetation types might be greater than fynbos species but they are able to release greater concentrations of leachates at a faster rate and contribute to the humic compounds pool in the soil. According to Northup et al., (1998) high polyphenol concentrations within plant material are expected in oligotrophic systems. The polyphenols are viewed as important regulators of the plant-litter-soil interactions.

Microbial activity within oligotrophic soils has its limitations. These limitations come in the form of the ability of the microbes to decompose humics such as polyphenols (Neuhauser and Hartenstein, 1978; Schimel et al., 1996) and the low levels of nutrients, which will affect their proliferation in terrestrial and aquatic systems (Alexander, 1977; Qualls and Richardson, 2000). Cleveland et al., (2002) found that microbial decomposition of organic carbon was severely reduced by low phosphorous content, concluding that the abilities of micro-organisms are potentially limited by P concentrations. From our fertilisation experiment (Figures 6 and 7) these limitations do appear to hold to a certain extent in nutrient poor soils. The N fertilisation within experiment 2 produced the lowest concentration of humic acid and indeed lowered the concentration in some soils. Therefore the N fertilisation provided a nutrient source for the microbes to partially proliferate and breakdown the humic acids within the soil. The limited ability of the microbes to have an effect of the humic compound pool will allow for greater amounts of carbon to chelate with metals and be leached into aquatic systems.

P fertilisation had the opposite affect to the nitrogen. The P and N+P fertilisations produced significantly higher humic acid concentrations compared to the other treatments. In the distilled water addition there was a significant difference between the two P treatments, however in the humic acid addition, the N+P treatment produced significantly less humic acid than the P treatment. Overall the phosphate in the P fertilisation appeared to bind to the polyphenols that were present within each soil type. Polyphenols in general have a high affinity to bind to proteins and inorganic ions such as
phosphate (Stewart and Wetzel, 1981). This affinity is repeated in the ability of root
exudates in the various forms to complex with metal phosphates during potential nutrient
acquisition (Dakora and Phillips, 2002; Lambers et al., 2003; Jones et al., 2003). The
prevalence of this interaction is greater in low pH environments, compared to alkaline
environments where phosphate and humic compound interactions are less likely to occur
(Stewart and Wetzel, 1981). Therefore the increase in humic acid concentrations within
all soils types with P fertilisation is a result of the humic acids within the soil readily
binding to the phosphate ions in the solution. This phenomena posses a new situation in
that if P levels were to increase in the oligotrophic soils they would be unavailable for
microbial consumption. At the same time, the already scarce P in the soils is liable to
eluviation and leaching out of the soil column. This situation could have serious knock on
affect on the P cycle as we know as more P is potentially being removed from the system.
However one could also look at it from the point of view that the polyphenols are
potentially mobilizing P for plant use.
Conclusion

In conclusion, four important chemical correlates are associated with the blackwaters of the South Western Cape, pH, organic carbon and iron. Each of these correlates is intricately linked to one another. The high organic carbon levels are a result of polyphenols from the high yielding sclerophyllous plants of the terrestrial environment. Fynbos species posses' high levels of polyphenols in their leaf material and the capacity to rapidly leach them. The polyphenols complex with the Fe in the soil column and in turn is leached out as organo-metal complexes. Polyphenols are not easily broken down by microbes and the low nutrient supply for he microbes adds to this dilemma, this allows for less decomposition to take place. However the role of polyphenols in an environment such as this can be viewed as key regulators of nutrient acquisition and conservation (Northup., et al 1998). This is clearly depicted in the P fertilisations where the polyphenols a have a high affinity for metal phosphates and readily bind to them. The situation adds to the low levels of available P for potential microbes to feed off. Interactions between the humic compounds and soil properties are complex .However, the lack of microbial activity and the high levels of humic compounds produced by the sclerophyllous vegetation of the South Western Cape both contribute to the final outcome of black verse white aquatic systems. In regions where aquatic systems are not dark, the vegetation and soil interactions prevent a large build up of humic compounds in the soil. The soil carbon that is available is not easily leached into aquatic systems.

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References


PLAGIARISM DECLARATION

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Figure 2: The correlations of total organic carbon, humic acid, iron, pH against the absorbance at 230 nm of the analysed river water samples.
Figure 3: The correlation between humic acid concentration of and the iron concentration in the analysed river water samples.

$\text{Fe (\text{mM})}$

$\text{Humic Acid (mg/l)}$

$r^2 = 0.3173$

$p < 0.05$
Figure 4: The concentrations of humic acid (± SE) leached from the plant material of 12 species and a bunch grass sample measured at 0.25, 0.5, 1, 2, 4 days during the in vitro experiment.
Figure 5: The rate of humic acid released (± SE) per gram dry plant material per day during the *in vitro* leaching experiment for all 13 specimens. The means of average ± SE, n = 3, for each vegetation type are indicated in brackets. Different letters denote the significance between vegetation types at p < 0.05 using Tukey LSD post hoc contrasts.
Figure 6: The concentrations of humic acid in each of the soils during experiment 1 (H₂O = X; N = ; P = A; N+P = ). The bar chart represents the mean humic acid concentration (± SE) calculated from the log transformed values. Different letters indicate the significance between treatments at p < 0.05 using Tukey LSD post hoc contrasts.
Figure 7: The concentrations of humic acid in each of the soils during experiment 2 (H2O = X; N = •; P = •; N+P = •). The bar chart represents the mean humic acid concentration (± SE) calculated from the log transformed values. Different letters indicate the significance between treatments at p < 0.05 using Tukey LSD post hoc contrasts.
Table 1: The five soils types used during the fertilization. Indicated are the vegetation types experiments found where the samples were collected including the results from the analyses for N, P and pH of each soil.

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<th>Longitude (E)</th>
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<th>pH</th>
<th>% N</th>
<th>P (mg/kg)</th>
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