

## Biogeochemical Processes and Implications for Nutrient Cycling

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### Summary

The availability and cycling of nutrients is determined by an interaction of physical, chemical, and biological processes in an ecosystem. This interaction of processes, collectively known as biogeochemistry, is important as it determines the forms, transformations, and ultimate fate of nutrients in a given system. This chapter focuses on biogeochemical processes in springs and spring runs with an examination of the role of springs as a potential ‘source’ or ‘sink’ for nutrients and pollutants.

### The Literature: What is Known

- The effect of biogeochemical processes on spring water composition begins immediately upon emergence from the boil and continues throughout the entire length of a spring run. In this way, the biogeochemical character of a spring and its biota depend greatly on the chemical nature of the groundwater source.
- Key interfaces involved in elemental cycling in springs environments are biofilms, algal mats and hyporheic sediment zones. These are areas of high biological activity and as such, have the potential to display indicators of major processes and nutrient effects (e.g., through enzyme activities).
- A variety of processes are known to occur in springs including organic matter decomposition, nitrogen fixation, nitrification, denitrification, iron reduction, sulfate reduction, and associated processes involving phosphorus. The relative importance of each process depends greatly on the conditions of individual springs.
- Current data have increased our understanding of nutrient sources, and have provided indications of some nutrient transformations such as overall system productivity, potential biotic uptake and transformation of inorganic nutrients, and potential losses of nutrients from the system (e.g., gaseous losses or sediment storage).
- Elevated levels of nitrate in Florida springs are currently a major concern, especially due to the suggested correlation of nitrate with increased algal growth. There is clear evidence of elevated nitrate levels in many springs; however, there is a high likelihood that the effect of increased nitrate may be coupled to other nutrient levels (e.g., phosphorus or metals) or physico-chemical parameters (e.g., dissolved oxygen).

## The Literature: What is Not Known

- Despite our understanding of biogeochemistry in other aquatic systems, there is little information regarding biogeochemical processes in spring systems where the focus has mainly centered on water column nutrients/water quality indicators. Consequently, our current biogeochemical models of Florida spring systems are more of a black-box with emphasis on water column nutrient levels and little understanding of individual processes or differences between systems of various types.
- There is little information to ascertain the involvement of processes/cycles controlling nitrate levels (e.g., plant uptake versus denitrification) or alternative pathways affecting nitrate conversion and loss (e.g., iron and sulfur involvement in lithotrophic denitrification).
- Among the studies which could advance our understanding of springs as sinks sources and transformers of nutrients are:
  - Expanding water quality data collection to include additional geochemical parameters (e.g., sulfide) could improve our ability to explain and predict individual spring responses to nutrients.
  - Increasing the frequency of water quality data collection (e.g., diel patterns) will offer additional insight into our ability to identify pathways involved in spring processing/cycling of nutrients.
  - Basing the collection of water quality data on stream segments will improve our ability to understand processes and spring run characteristics contributing to nutrient uptake and transformation.
  - Characterization of sediments (e.g., nutrient storages, organic matter distributions, mineral composition) would enable better assessments of benthic and hyporheic processes affecting both existing and future water quality (sediment memory).
  - Aquatic plant management efforts are likely to result in significant alteration of the spring environment through episodic deposition of organic matter, however, as yet there are no studies to document the potential for these activities to alter biogeochemical conditions (i.e., creating anoxic zones) and cycles (e.g., increasing denitrification loss of nitrate or increasing release of ammonium and phosphorus).
  - More studies are needed to document the involvement of mechanisms in observed declines in nitrate with distance in spring runs (i.e., dilution vs. biotic uptake vs. denitrification).
  - More exploratory work is necessary to determine the existence and importance of novel pathways of nitrate conversion (e.g., anammox and lithotrophic nitrate reduction).

## INTRODUCTION

In combination with light, nutrient availability is a dominant factor controlling aquatic productivity. For this reason, nutrient levels, transformations, and fates are key factors in determining the health of aquatic resources such as spring ecosystems. Increased levels of nutrients in many spring systems are also a growing concern, particularly in Florida where a significant portion of the groundwater interacts with karst geology. This interaction gives a unique character to Florida springs as ecosystems, and has important implications for the ways in which these systems respond to, process and cycle nutrients.

The study of biogeochemistry deals with the interaction of biological systems with geochemical reactions which determines the overall cycling of elements within ecosystems. Both abiotic and biotically-controlled geochemical reactions can affect nutrient and mineral solubility, as well as reduction/oxidation (redox) chemistry, which can affect the composition and activity of the biotic system which in turn affects the water chemistry. Whether a spring system is a source or sink for nutrients and pollutants in the landscape is determined by the coupled biogeochemical processes occurring in its water column, sediments, biota, and adjacent floodplains.

The potential biogeochemical reactions and pathways occurring in springs are numerous, and have been studied in varying levels of detail. Therefore the purpose of this review is 1) to describe the biogeochemical processes related to nutrient cycling in springs or similar systems, 2) summarize the available literature for Florida spring systems, and 3) highlight the needs and directions for future spring research. The major literature sources for this review were derived from published journal articles and government/agency reports. These documents included geochemical studies, biological/microbiological studies, and ecosystem/ecology studies focused on spring systems including those from Florida and other springs around the world. An overall lack of spring-specific data on many processes forced the use of literature pertaining to similar systems, such as chalk and other limestone-based streams. Finally, general stream literature was also used where appropriate to describe similar processes occurring in lotic (flowing) systems.

## SPRING ENVIRONMENTS/ORGANISM GROUPS

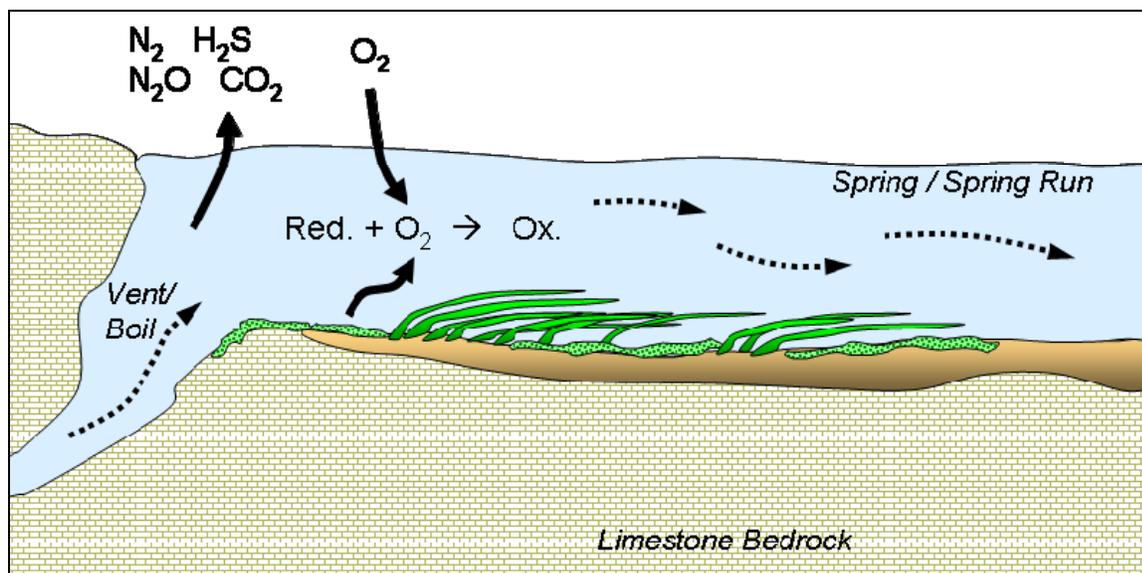
### Spring water chemistry

Water chemistry is one of the most important characteristics of a spring system which directly impacts its biological functioning. Because the primary source of spring water is groundwater, the characteristics of the different aquifers such as mineralogy and residence time will determine the overall chemical character of the spring receiving this outflow. Slack and Rosenau (1979) classified Florida springs into four types, in order of occurrence: 1) calcium-magnesium bicarbonate, 2) sodium chloride, 3) mixed (no dominant cation or anion) and, 4) calcium sulfate. This review will focus on the dominant spring water type in Florida - the hard water type, which has high calcium-magnesium-bicarbonate concentrations resulting from the interaction with limestone and dolostone host rock. However, other chemical constituents characterizing the other spring water types will also be addressed as they relate to specific processes.

Groundwater confined within the aquifer for extended periods of time undergoes significant changes as it emerges at the spring vent or boil and is directly introduced into conditions at the earth surface. Groundwater (at more or less equilibrium with subsurface temperature/ pressure/ mineral compositions) changes dramatically when introduced into the high oxygen/reduced pressure environment at the boil/vent/or seep (Figure 1). In general, soil/aquifer respiration processes increase the partial pressure of CO<sub>2</sub> in ground waters, which in limestone systems is responsible for the dissolution of the aquifer bedrock to form karst features. The equilibrium reaction for this process is given by the equation:



Dissolution of limestone and dolostone results in high levels of alkalinity (carbonate buffering) and dissolved constituents (e.g., calcium, magnesium, chloride, sulfate) which give rise to the chemical composition of most spring waters in Florida.



**Figure 1** – Depiction of degassing and oxidative conversion of chemically reduced species (Red.) to oxidized (Ox.) forms following emergence of groundwater in a spring vent/boil.

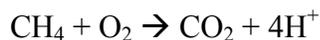
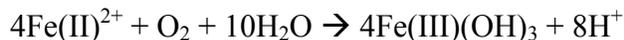
Emergence of groundwater at a boil or vent results in an immediate disequilibrium of the water with respect to CO<sub>2</sub>, and as a result, degassing occurs. Loss of excess CO<sub>2</sub> shifts the dissolution equation toward precipitation of CaCO<sub>3</sub> which results in supersaturation of groundwater with respect to CaCO<sub>3</sub> (Dreybrodt *et al.* 1992). Other gaseous constituents of groundwater have the potential to degas upon emergence including N<sub>2</sub> (produced through aquifer (denitrification), the greenhouse gas N<sub>2</sub>O from (from incomplete nitrification/denitrification processes), and caustic H<sub>2</sub>S from sulfate reduction (evidenced by a rotten egg smell).

Supersaturation of CaCO<sub>3</sub> may further result in CaCO<sub>3</sub> precipitation and mineral formations near boil or seep areas (Drysdale *et al.* 2002; Liu *et al.* 2006). This phenomenon is not common in Florida, but the process of CaCO<sub>3</sub> precipitation can result in mineral encrustation in localized

areas of some springs, especially where processes such as photosynthesis further enhance supersaturation through uptake of CO<sub>2</sub>. This process can result in mild calcifications on surfaces of submersed aquatic vegetation (SAV) or algae filaments (Berrie 1992).

If sufficient amounts of organic materials are present in the groundwater, respiration processes within confined aquifers result in a depletion of available oxygen potentially creating anaerobic conditions. Many springs report dissolved O<sub>2</sub> levels <1 mg L<sup>-1</sup> (Scott et al. 2004). Under anaerobic conditions, other oxidized species (e.g., NO<sub>3</sub><sup>-</sup>, Fe(III), and SO<sub>4</sub><sup>2-</sup>) can become chemically reduced (forming e.g., NH<sub>4</sub><sup>+</sup>, Fe(II), S<sup>2-</sup>). In extreme cases, the highly reduced gas, methane (CH<sub>4</sub>) can even be formed. As a result, many groundwaters can contain a number of reduced species under low-oxidizing conditions. These reduced species are highly reactive, however, they can also persist in groundwaters (even under aerobic conditions) for significant periods of time (Darling and Goody 2006).

At the boil, chemically reduced spring waters encounter atmospheric levels of O<sub>2</sub> leading to a suite of chemical oxidation reactions:



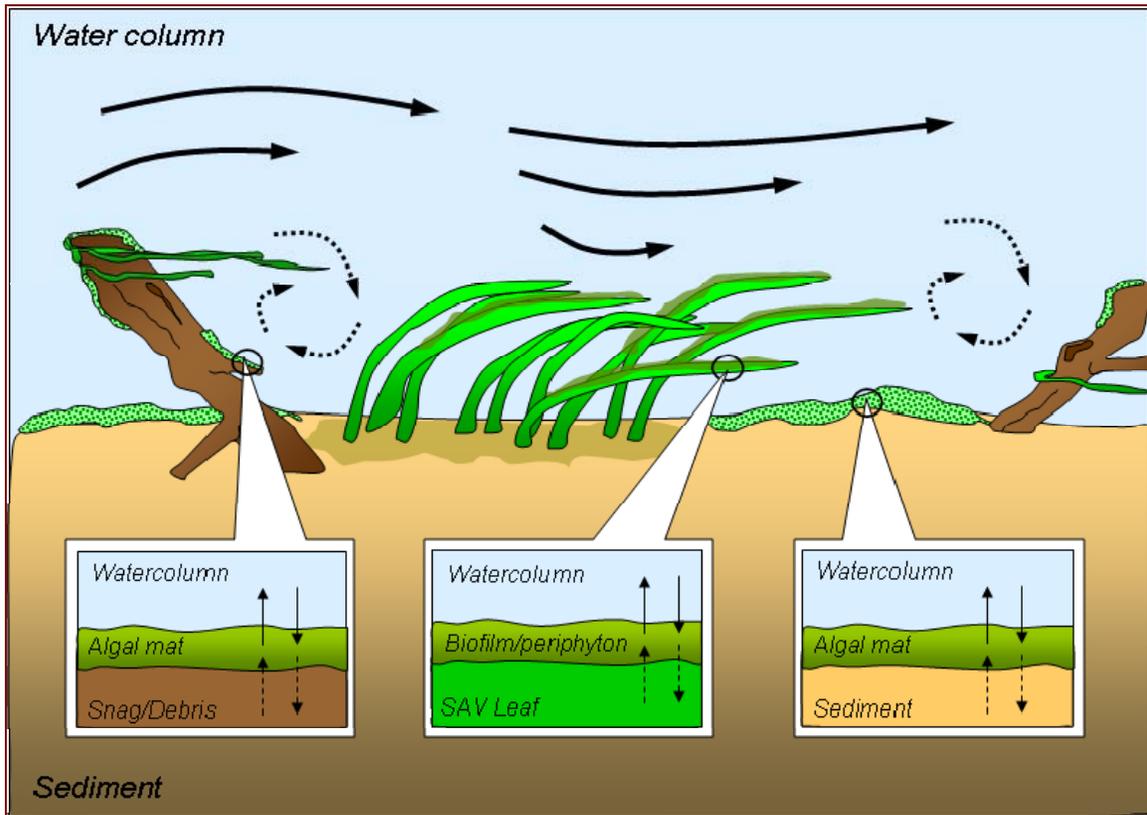
Two important consequences of each of these oxidation reactions include: 1) consumption of O<sub>2</sub> and 2) the production of H<sup>+</sup> ions. Depending on the redox status of the groundwater, consumption of oxygen may continue even after springwater emergence. As groundwater species are oxidized, the resulting acidification also increases the potential for dissolution of CaCO<sub>3</sub> (in systems supplied with highly reducing groundwater) while degassing and photosynthesis (reducing CO<sub>2</sub> levels) results in precipitation of CaCO<sub>3</sub> (Guasch *et al.* 1998). The balance between these two extremes determines the extent of calcification processes which is an important determinant of vegetative species composition (particularly algal assemblages) in spring systems (Freytet and Verrecchia 1998).

## Reactive Zones

The high energy, lotic characteristics of springs create a variety of different zones where biogeochemical reactions can occur. The most important zones are the interfaces of the water column with sediments, epilithic/periphytic biofilms, and benthic mats of macroalgae (Figure 2). These interfaces are critical to nutrient cycling processes because of the sharp gradients they create in physical and chemical parameters (e.g., pH, O<sub>2</sub>, redox). In addition, solutes are actively produced and consumed creating concentration gradients which further enhance solute movement and transport.

## Biofilm

In aquatic systems, development of structural microbial assemblages, sometimes referred to as biofilms, is influenced by the habitats which may be present. In a stream or a spring run, the presence of rocks and stones, litter, detrital material, logs, plants and other submerged surfaces provide substrates for the microbial biofilms to establish. In well-developed stages, aquatic biofilms are known by names such as periphyton or aufwuchs.



**Figure 2** – Depiction of biogeochemical exchanges occurring at the biofilm, epiphyte, and benthic algal mat surfaces in a spring system.

Biofilm forming algae and bacteria actively exude substantial quantities of exopolymeric substances (EPS) into the environment that are primarily organic carbon (Hoagland *et al.* 1993; Costerton *et al.* 1995; Goto *et al.* 1999). This exudation of carbon can constitute a large proportion of carbon acquired by both bacteria and algae (Goto *et al.* 1999) and result in the accumulation of non-living organic carbon on surfaces in aquatic environments (Wetzel, Ward & Stock 1997; Frost & Elser 2002).

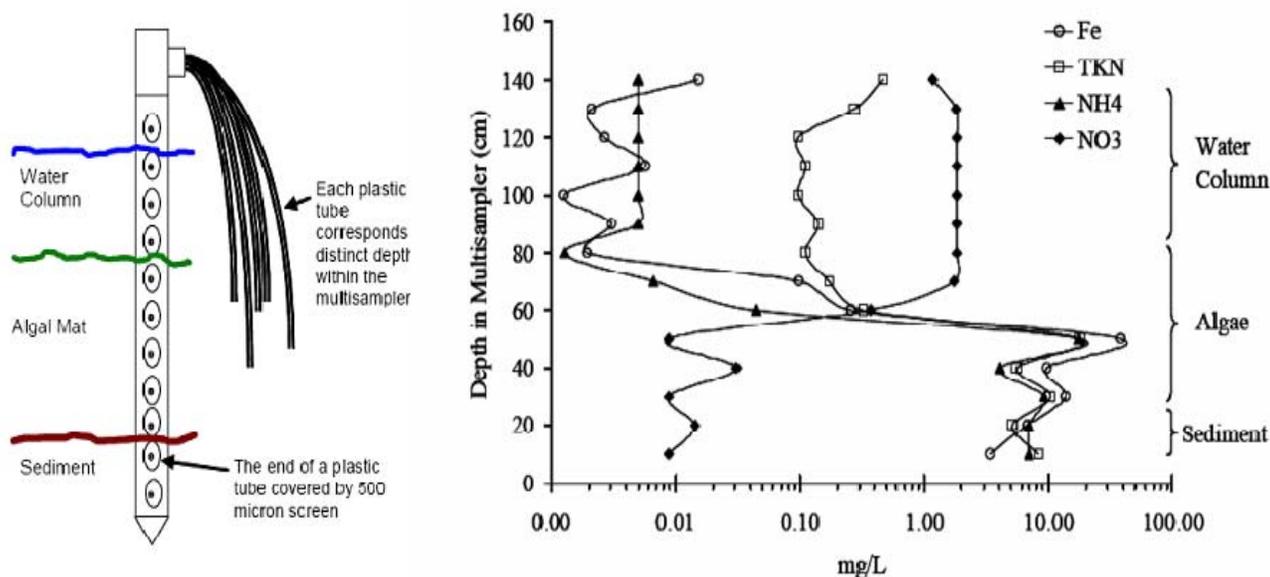
In aquatic systems like springs, biofilms are dominated by algae, diatoms, bacteria, and fungi. Exuded C provides the basis for other heterotrophic organisms to colonize, and as the biofilm develops, respiration can consume available oxygen to create anaerobic zones within the biofilm. The creation of anaerobic microsites and layers allow a myriad of other bacterial groups to become established. These groups represent important classes of biogeochemical processes

including denitrifiers, other anaerobic (iron reducers, sulfate reducers), and other aerobic organisms (nitrifiers, iron/sulfide oxidizers).

### **Benthic Algal Mats**

Conspicuous mats of benthic algae are becoming a common feature of many spring systems (Stevenson *et al.* 2007). These macroscopic algal growths, similar to biofilms, are actually communities of microorganisms including: algae, bacteria, and fungi. The initial formation of mats is usually based on the structure provided by filamentous algae, which provide the framework for accretion of other organisms (e.g., diatoms, bacteria) and additional organic and inorganic materials (Stal 2000). The two most common algal taxa forming these mats in Florida systems are *Lyngbya* and *Vaucheria*, however a variety of other filamentous or colonial species can initiate mat formation (Stevenson *et al.* 2007).

As the algal filaments develop, accretion progresses, and secondary organisms begin to dominate and alter the mat's physical and chemical characteristics. Physical gradients (light, temperature) form as light penetration to deeper mat layers becomes limited (Jorgensen *et al.* 1983). In response to the physical gradients, photosynthetic mat organisms become structured (or in some species, migrate vertically) to maximize their location in the presence of light and temperature. (Stal 2000). With the localization of C fixation, nutrient gradients develop in response to increased autotrophic and subsequent heterotrophic growth. Gradients of reduced and oxidized compounds also form and drive mat structure and metabolism (Figure 3) (Pinckney and Paerl 1997; Stevenson *et al.* 2007). These gradients of nutrients and redox can occur vertically or in response to diel fluctuations of photosynthetically-induced oxygen, pH and C production. As a result, the character (i.e., species composition and physical characteristics) and associated functions of an algal mat can be drastically different both vertically and temporally within the mat.



**Figure 3** – Illustration of the sampling device and example of the measured profiles of nutrients from the study of benthic macroalgal mats by Stevenson, *et al.* (2007).

## Sediments

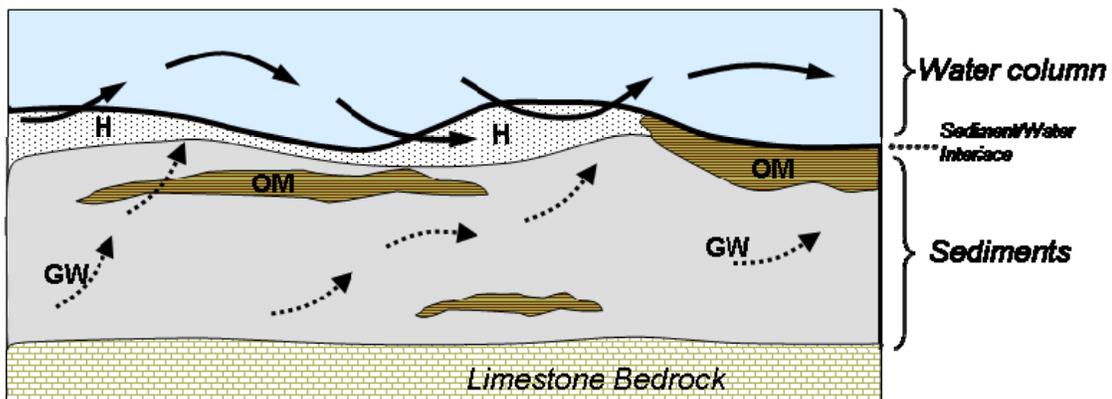
In addition to a dissolved load, springs from carbonate aquifers have also been shown to be significant sources of sediments (Herman *et al.* 2006). In aquifers based in marine deposits (such as in Florida) these particles may be derived from the direct surface connections with underground conduits, or from particles release through erosion or dissolution of the aquifer material itself. As a result, sediments in spring flows can vary in composition from small organic materials, to clay particles, to sands and carbonate minerals (Herman *et al.* 2006; Mahler and Lynch 1999). The direct transport of surface derived materials also results in a strong dependence of sediment flux at the boil on the springshed characteristics and relationships between rainfall, runoff, recharge, and discharge. For example some springs, normally clear under low flow conditions, become turbid after storm events.

Not much is known about the composition and distribution of sediment types in Florida springs and spring runs, including information regarding particle size, mineralogy, or organic content. To our knowledge, only one study has attempted to characterize sediments/subaqueous soils from a spring system (Saunders 2007). In other stream environments, flow velocity is the most important determinant of sediment distribution patterns, therefore, it is likely the same would hold true for springs with flow velocities ranging from ca 1 to 100 cm s<sup>-1</sup>. In general, particle sizes of sediment should decrease with passage downstream as channel widths widen, current velocity decrease, and progressively finer size classes are allowed to settle out. Saunders (2007) confirmed this pattern for the upper Chassahowitzka River where the areas near the boil were dominated by sands low in organic matter, and areas further downstream consisted of finer sands with higher organic content.

The main biogeochemical feature of spring run sediments is the presence or absence of redox gradients. In sediments, microbial respiration frequently consumes available O<sub>2</sub> leading to the formation of anaerobic zones at depth. Under these conditions O<sub>2</sub> contained in the water will diffuse downward to the anoxic sites, creating a gradient of O<sub>2</sub> and the presence of both aerobic and anaerobic sediment zones. In aerobic zones, microbes are involved in biogeochemical reactions and use oxygen as the primary electron acceptor, while in anaerobic layers, facultative and obligate anaerobes function using alternate electron acceptors (e.g., NO<sub>3</sub><sup>-</sup>, Fe(III), and SO<sub>4</sub><sup>2-</sup>) in their respiration.

One feature of sediments in high energy lotic systems is the high percentage of coarse particle size classes (e.g., sands) which greatly enhances advective processes (Figure 4). Sediments of high energy spring runs are also more likely to include varying stratigraphies with layers of different composition (e.g., organic matter) juxtaposed. In a manner analogous to that in biofilms or benthic mats, these types of sediment distributions serve to enhance the potential for varying reactions where reactants and products are more easily exchanged between reactive zones to greatly increase reaction rates (Harvey and Wagner 2000).

Characteristics of each of the sediment habitats in spring systems give rise to a variety of functional groups of organisms. Varying redox and nutrient conditions in sediments of springs likely result in the coexistence of both aerobic and anaerobic genera. This increased diversity of organisms and metabolisms should enhance the potential biogeochemical cycling of nutrients such as carbon, nitrogen, and phosphorus. Bacteria, in particular, are constantly responding and adjusting to carbon and nutrient availability, and thus, their activities can indicate conditions of availability. One of the ways microorganisms react to environmental conditions is through expression of enzymes which play a pivotal role in catalyzing the rate limiting steps of biogeochemical processes. For this reason, enzyme assays can serve as excellent indicators of microbial activities and environmental conditions (e.g., nutrient/contaminant impacts) of aquatic systems (Chrost 1991; Sinsabaugh *et al.* 1997).



**Figure 4** – Diagram of characteristics and hyporheic flow processes related to biogeochemical functions in a hypothetical spring run sediment profile. H, hyporheic zone; OM, organic matter concentration; GW, groundwater movement.

## BIOGEOCHEMICAL PROCESSES

### Carbon

As the major component of all life, carbon is arguably the most important of all biological elements in ecosystems. The cycling of C in spring systems has important implications for productivity, food-web dynamics, and metabolism of all spring biota. Consequently, the biogeochemical cycle of carbon involves complex interactions between and within organic and inorganic C reservoirs and involves numerous metabolic pathways and organism groups. These interactions have been widely studied by scientists in various disciplines.

### Carbon Sources

The main sources of C to a spring system occur as 1) C sources in the groundwater; 2) C from direct or diffuse inputs of surface or groundwater along the spring run; 3) C inputs derived from riparian vegetation (litterfall); and 4) C fixed into biomass of spring autotrophs (primary producers). Of these, the C derived from autotrophic production within the spring run would be considered autochthonous (within-system) sources while the remainder are C sources external to (or allochthonous to) the spring run.

In aquatic systems, carbon is predominantly present as dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), and as particulate organic (POC) and particulate inorganic (PIC) carbon forms. DIC is the primary form of C in hard water (limestone-based) springs, and is the primary form of C available for photosynthesis. For this reason, DIC is rarely limiting to autotrophic production in springs such as those in Florida. In contrast, ground waters in karst systems generally have low concentrations of DOC, and thus, much of the secondary production in springs is likely derived from autochthonous sources of C either directly, or following decomposition (Berrie 1992).

Perhaps one of the largest sources of C to spring systems is through the connections of many spring runs with extensive floodplains with forested, high-C soils (Whiting and Stamm 1995). This becomes significant during high flow periods which could result in significant exports of floodplain particulate C or leaching of C and subsequent increases in spring DOC concentrations. Hydrologic fluctuations (i.e., variations in rainfall and spring flow/discharge) can also lead to the introduction of variable amounts of high DOC surface water into groundwater systems (Pronk et al. 2006). Under high flow conditions there are also greater inputs of C from connected streams and discharges of surface or drainage waters.

As they are known to actively exude C compounds, both algae and SAV are likely important as autochthonous C sources in springs. These exuded compounds can range in size from low molecular weight (LMW) compounds such as sugars, proteins, amino acids, enzymes, phenolics, glycolate, and alkaloids (Sondergaard 1983) to high molecular weight (HMW) compounds (>10,000 Daltons, where a dalton  $\approx$  the mass of one hydrogen atom) including primarily polysaccharides which can appear as slimes or algal sheathes (De Philippis and Vincenzini 1998; Stal 1994; Wetzel and Penhale 1979). The composition and production of extracellular C is

specific to both organism type and growth conditions including culture age, N source, temperature, salinity, P, and potassium (reviewed by De Philippis and Vincenzini 1998). Net release rates of 5-10% of total carbon fixation in macrophytes have been observed (Wetzel and Penhale 1979), while in some species of cyanobacteria, extracellular C production can represent up to 50-70% of dry weight (Bertocchi et al. 1990). Production of EPS has also been shown to increase with increasing irradiance in algal cultures (Otero and Vincenzini 2003; Renstrom and Bergman 1989). For this reason, light availability, and rates and patterns of algal photosynthesis/ photorespiration, can be a significant determinant of the quantity and quality of C compounds released within a spring system.

When present, EPS is considered a bioavailable C source that can stimulate bacterial respiration and productivity (Neely and Wetzel 1995; Espeland *et al.* 2001). Consequently, microbial associations have been shown to alter the composition of products excreted by the source plant (Sondergaard 1983). Overall, exuded C sources are viewed as highly available, particularly for the microbes associated with the algal cells or SAV leaves. In order to utilize exuded C substrates, bacteria must hydrolyze complex polymeric OM into simple utilizable forms by producing extracellular enzymes, in particular glucosidases (Chrost 1989; 1991; Eivazi and Tatabai 1988; Arsnoti 2003).

## **Decomposition**

In the absence of direct exudation of C compounds, secondary production is based on C derived through decomposition. In most aquatic systems, this is the dominant pathway of C flow, and is the primary influence on the metabolic activity and diversity of microbes conducting many other biogeochemical processes. Springs are not known as accumulators of organic matter, perhaps due to their high flow velocity and (in most cases) continuous supply of well-oxygenated water. Temperature is seasonally controlled (20-24 °C), and so decomposition should occur at a moderate rate throughout the year. Also, the well-buffered hard waters of most spring systems should prevent low pH conditions from inhibiting microbial decomposition activity as in acid bogs.

As in other lotic systems, organic matter in springs tends to accumulate in dense vegetation stands, debris dams, and at the bottoms of large pools. In a slow moving spring run (upper Chassahowitzka River), Saunders (2007) observed large (>5 cm) organic material horizons with a patchy distribution. Similarly, in chalk streams, seasonal changes in beds of aquatic vegetation were shown to strongly affect flow velocities and rates of sediment and organic matter accumulation (Cotton *et al.*, 2006). For this reason, seasonality and management of SAV can have a direct impact on distribution of zones of organic matter accumulation and the biogeochemistry of a spring system. Aquatic vegetation management, including spraying of herbicides (e.g., Wakulla Springs State Park) can also contribute a significant amount of C (in large pulses) to spring runs.

Decomposition (or C mineralization) is a complex process beginning with the physical breakdown of macroscopic plant/algae tissues. The major constituents of this detrital plant matter are soluble substances (such as sugars, fatty acids, and amino acids), structural compounds (such as cellulose, hemi-cellulose, lignin and associated proteins), other water-

insoluble components (including fats, waxes and resins), and mineral matter (ash). The relative proportion of these constituents varies with type and source of detrital matter, degree of decomposition, and the age of the material. While SAV in springs contain more soluble and less structural C compounds than vegetation in the riparian zone, algae contain even fewer structural compounds. Approximate ranges in values for these constituents in plant material, peat, and soil organic matter are shown in Table 1 (Reddy and DeLaune 2008).

The initial step in the decomposition process is leaching of water soluble organic and inorganic compounds as a result of cell autolysis. Water soluble components include soluble carbohydrates, nucleotide bases, fatty acids, and amino acids. These components are readily bioavailable and serve as energy and nutrient sources to bacterial and fungal communities colonized on detrital plant tissue. Physical leaching of soluble components is generally complete within a few weeks of plant senescence, depending on structural components, temperature, and water flow. Leaching can be a significant factor in decomposition of non-lignocellulose materials such as algal mats and SAV (>50% mass loss). However, in detrital tissue containing lignocellulose materials (such as tree leaves and woody materials) only approximately 10-20% is lost within four weeks after immersion in the water column (Benner *et al.* 1985).

**Table 1.** Major carbon components of plant tissue, peat, and soil organic matter.

| Component                                      | Plants        | Peat  | SOM   | Monomer                        | Linkage                             |
|--|---------------|-------|-------|--------------------------------|-------------------------------------|
|  | % composition |       |       |                                |                                     |
| Cellulose                                      | 15-60         | 15-30 | 2-10  | Glucose                        | $\beta$ -1,4 ether                  |
| Hemi-cellulose                                 | 10-30         | 10-40 | 0-2   | 5C & 6C sugars                 | $\beta$ -1, 4 & $\beta$ -1, 3 ether |
| Lignin   | 5-40          | 20-60 | 30-50 | Phenyl propane                 | Aryl ether & C-C                    |
| Water soluble fatty acids, sugars, amino acids | 10            |       | <1    | NA                             | NA                                  |
| Ether extractable lipids, oils, waxes, etc.    | 1-8           | 2-10  |       | fatty acids, sugars, phosphate | Esters & C-C                        |
| Proteins                                       | 2-20          | 4-20  | 2-20  | amino acids                    | Peptide                             |
| Mineral matter                                 | 1-10          | 5-25  |       |                                |                                     |

After initial leaching and fragmentation, breakdown of plant detritus depends on the amount of major polymers, including lignin, cellulose, hemi-cellulose, lipids and waxes, and proteins. These structural components are mainly particulate in nature and must be broken down. In other aquatic systems, macroinvertebrate colonization is a key factor affecting observed litter decomposition rates; however, studies of their effect in springs are few and inconclusive. For example, in a study of four springs in Minnesota, Bortodziej and Perry (1990) found similar rates of decomposition of watercress (*Nasturtium officianale*) in different spring types despite significant differences in macroinvertebrate colonization. In the same study, they also found that macroinvertebrate colonization was correlated with decay rates of Boxelder (*Acer negundo*). Results such as these may indicate that macroinvertebrate shredder and grazer function may

depend on plant biomass type/quality (e.g., high versus low contents of structural compounds) in high energy spring environments.

Decomposition of particulate matter into smaller particles is an important process in determining C availability for secondary production and biogeochemical metabolism in spring systems. Plant detritus (either attached or detached from the plant) undergoes physical fragmentation through the action of waves and currents, UV exposure, and through grazing activities of macroinvertebrates (Dawson 1980). Fragmentation of plant detritus into fine particulate matter (FPOM) fraction (<1 mm) results in increased surface area, and accelerated microscale processes (such as enzymatic hydrolysis and catabolic activities). Coarse particulate matter (CPOM) being reduced into smaller fractions greatly enhances the surface area which fungi and bacteria can attack. In lotic environments, the action of macroinvertebrate shredders results in a transport of C particulates from the headwater reaches and a steady accumulation of fine particulate organic matter (FPOM) at lower stream zones (Webster *et al.* 1999). As it travels downstream, this FPOM material becomes increasingly more bioavailable through continued decomposition and the incorporation of added nutrients and algal derived particulates (Allan and Castillo 2003).

Even FPOM must first be converted into smaller metabolizable units before it can be assimilated by microbes as energy and nutrient sources. Extracellular enzymes secreted by microbes (bacteria and fungi) aid in hydrolysis of high molecular weight organic compounds associated with plant detritus and soil organic matter, which in their original form are too large for transport into the periplasmic space of the cell (Weiss *et al.* 1991). Microorganisms including bacteria and fungi are the dominant producers of extracellular enzymes. Selected examples of enzymes involved in degradation of cellulose, hemi-cellulose, and lignin are shown in Table 2. Breakdown of these structural polymers may require a multicomponent enzyme system including a wide range of microbial species. Rarely can a single microorganism produce all the enzymes required for breakdown of organic substrates.

Extracellular enzymes are generally considered as the rate limiting step in the overall degradation of organic matter. Kinetic limitations to enzymatic breakdown include slowing diffusion of enzymes and substrates, adsorption of enzymes on solid phases, and complexation of enzymes to humic substances (Sinsabaugh 1993; Wetzel 1991). Complexation of humic substances with enzymes may not be a significant issue in hard water springs as major cations (e.g.,  $\text{Ca}^{2+}$ ) react with humic acids and reduce the precipitation and inactivation enzymes. In soft water with low levels of base cations, the interference of humic substances on enzyme activities may be greater than hard water with  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  concentration in the range of 40-60 and 15-25  $\text{mg l}^{-1}$ , respectively (Wetzel 1991).

**Table 2.** Enzymes involved in the degradation of polymeric C compounds (Sinsabaugh et al. 1991). EC number = Enzyme commission number.

| Process                    | Enzymes                        | EC number   | Substrates |
|----------------------------|--------------------------------|-------------|------------|
| Starch degradation         | $\alpha,\beta,\gamma$ -amylase | 3.2.1.1,2,3 | Amylose    |
| Cellulose degradation      | Exocellulase                   | 3.2.1.4     | Cellulose  |
|                            | Endocellulase                  | 3.2.1.9.1   | Cellulose  |
|                            | $\beta$ -glucosidase           | 3.2.1.2.1   | Cellobiose |
| Hemi-cellulose degradation | Exoxylanase                    |             | Xylan      |
|                            | Endoxylanase                   | 3.2.1.8     | Xylan      |
|                            | $\beta$ -xylosidase            | 3.2.1.37    | Xylobiose  |
| Lignin degradation         | Phenol oxidase                 | 1.10.3.12   | Lignin     |
|                            | Peroxidase                     | 1.11.1.7    | Lignin     |

Because enzyme activity is the rate limiting step in microbially mediated decomposition of plant detritus in wetlands and aquatic systems, many researchers have studied enzyme activities over scales ranging from the molecular to the ecosystem level (Sinsabaugh *et al.* 1993). Activities of  $\beta$ -Glucosidase, endocellulase and exocellulase have been measured as indicators of organic matter decomposition in wetlands and aquatic systems. Activity should be on a relative basis, when comparing the results from various ecosystems. Despite their use in other aquatic systems, there has not been any published report of carbon degrading enzyme expression in Florida springs.

As decomposition progresses, soluble or easily hydrolyzed compounds are selectively degraded yielding increased recalcitrance with age of detritus. Decomposer biomass is also incorporated as populations wax and wane. In this way, organic matter in waters and sediments can be viewed as a complex of plant, microbial, and animal products in various stages of decomposition. Continued chemical alteration of the C mixture ultimately results in the formation of highly recalcitrant C classes including humic and fulvic acids (Stevenson 1994). This process of stable organic matter formation results in aquatic systems serving as accumulators of C, and in springs, this process can be accentuated by increased deposition and burial of organic matter by sediments.

## Nitrogen

Along with P, the high abundance of N in living biomass makes it one of the most highly demanded nutrients in aquatic systems. Nitrogen levels in many springs statewide have shown steady and dramatic increases over the past half century. In many cases, this excess N could result in significant changes in productivity; however, not all springs have exhibited changes with increasing N concentrations. The high level of N in some springs systems is also cause for significant concern (even if not related to within-spring impacts) from toxicological and landscape perspectives.

Numerous transformations and a suite of enzymes are involved in the transformation of N in aquatic systems (Table 3, Figure 5). Nitrogen is present in aquatic systems in organic forms and as inorganic forms such as dinitrogen gas (N<sub>2</sub>), nitrate (NO<sub>3</sub><sup>-</sup>), nitrite (NO<sub>2</sub><sup>-</sup>), ammonium (NH<sub>4</sub><sup>+</sup>), and trace gases such as N<sub>2</sub>O. Organic N is derived primarily from amino-bonded C (-NH<sub>2</sub>) such as amino acids and proteins, and nucleic acids, amino sugars. Generally, the sum of NO<sub>3</sub><sup>-</sup>/NO<sub>2</sub><sup>-</sup>, and NH<sub>4</sub><sup>+</sup> measured after filtration is taken to represent the dissolved inorganic N (DIN). The difference between the total dissolved N of a system and its DIN is then defined as the dissolved organic N (DON).

**Table 3.** Enzymes involved in various nitrogen cycle processes. From Reddy and DeLaune (2008).

| Process                 | Enzymes               | EC number | Substrates                   |
|-------------------------|-----------------------|-----------|------------------------------|
| N <sub>2</sub> fixation | Nitrogenase           | 1.18.6.1  | N <sub>2</sub> gas           |
| Amino acid degradation  | Amidase/<br>deaminase | 3.5.1.4   | monocarboxylic acid<br>amide |
| Amino acid degradation  | L-Glutaminase         | 3.5.1.2   | L-glutamine                  |
| Urea degradation        | Urease                | 3.5.1.5   | Urea                         |
| Nitrate reduction       | Nitrate reductase     | 1.7.99    | nitrogenous substrates       |
| Protein degradation     | Peptidase             | 3.4.21    | Amides                       |

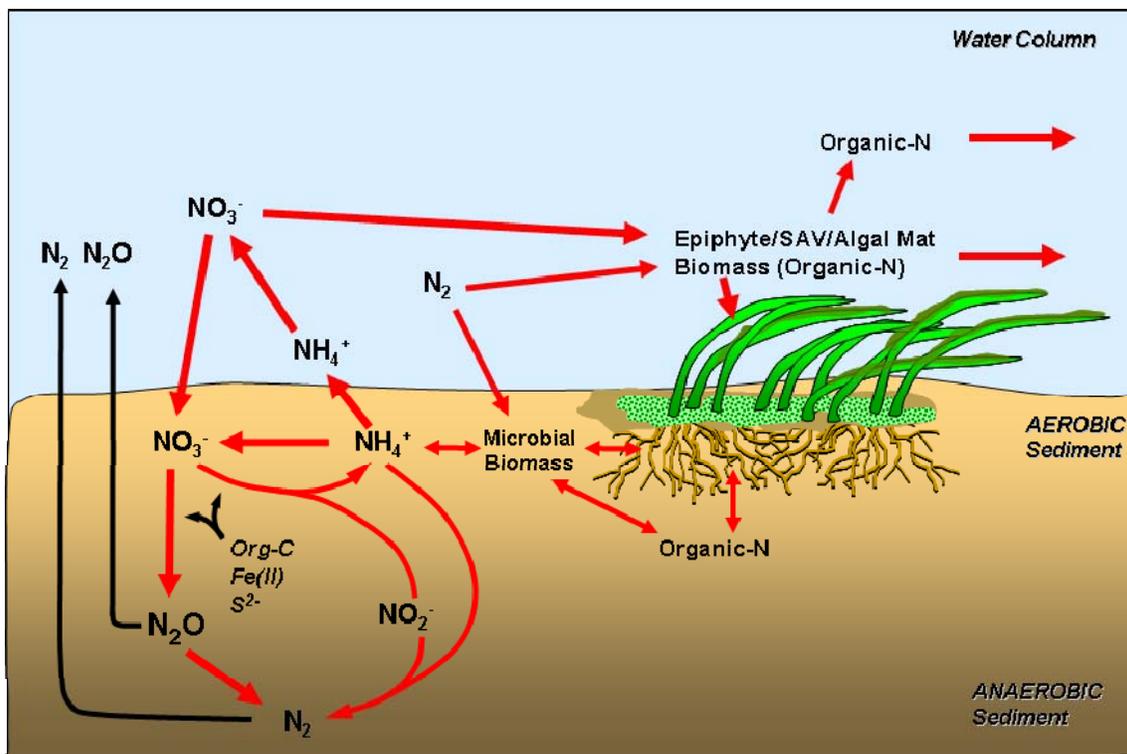
### Biological Nitrogen Fixation

Nitrogen is unavailable in the gaseous N<sub>2</sub> form and must be first converted (or fixed) to become bioavailable. One of the most studied enzymes (nitrogenase) is responsible for this conversion of N<sub>2</sub> gas to NH<sub>4</sub><sup>+</sup>, and is possessed by only select prokaryotic organisms including bacteria and cyanobacteria. Both of these groups are present in the biofilms, sediments and benthic algal mats of spring systems, however the potential for their expression of nitrogenase is uncertain.

A variety of factors can influence expression of nitrogenase enzymes including species composition, light availability, availability of P and metallic co-factors (Fe, Mo), oxygen levels, and NH<sub>4</sub><sup>+</sup> concentrations. In the case of the springs, NH<sub>4</sub><sup>+</sup> levels are generally not high, and should not be sufficient to suppress nitrogenase. Metal availability (in particular trace elements required for nitrogenase function) may be limiting in some springs; however, there are little data to support this conclusion. The presence of cyanobacteria in spring systems indicates that N-fixers may have a competitive advantage for N acquisition. It is true that many springs are N-limited, therefore, it is also likely that active N<sub>2</sub> fixation may be occurring. This has been documented in a number of stream systems; however, no data exist to ascertain the significance of this process for Florida springs. Furthermore, it is also uncertain what the fate of this fixed N would be in the spring environment.

## Nitrogen uptake

In streams and springs with high biomass and productivity, nitrogen required for growth that is not provided by nitrogen fixation must be supplied by N uptake and assimilation from the water column. High production of algae and SAV in Florida spring runs should also contribute to high N assimilation rates especially in areas where growth is N limited (Notestein et al., 2003; Inglett et al., 2007). Of the two inorganic dissolved N forms, both  $\text{NO}_3^-$  and  $\text{NH}_4^+$  are bioavailable and have been shown to be rapidly taken up by biota in headwater streams. Much of this headwater uptake is likely due to the high abundance of organic matter (leaf litter) in these systems. In one study of a low organic matter, brackish portion of the Chassahowitzka River, Saunders (2007) calculated rates of N loss (as  $\text{NO}_3^-$ ) from the water column as high as  $5 \text{ mg m}^{-2} \text{ d}^{-1}$ , however; it was not determined what proportion of this N loss was due to biotic uptake, denitrification, or dilution.

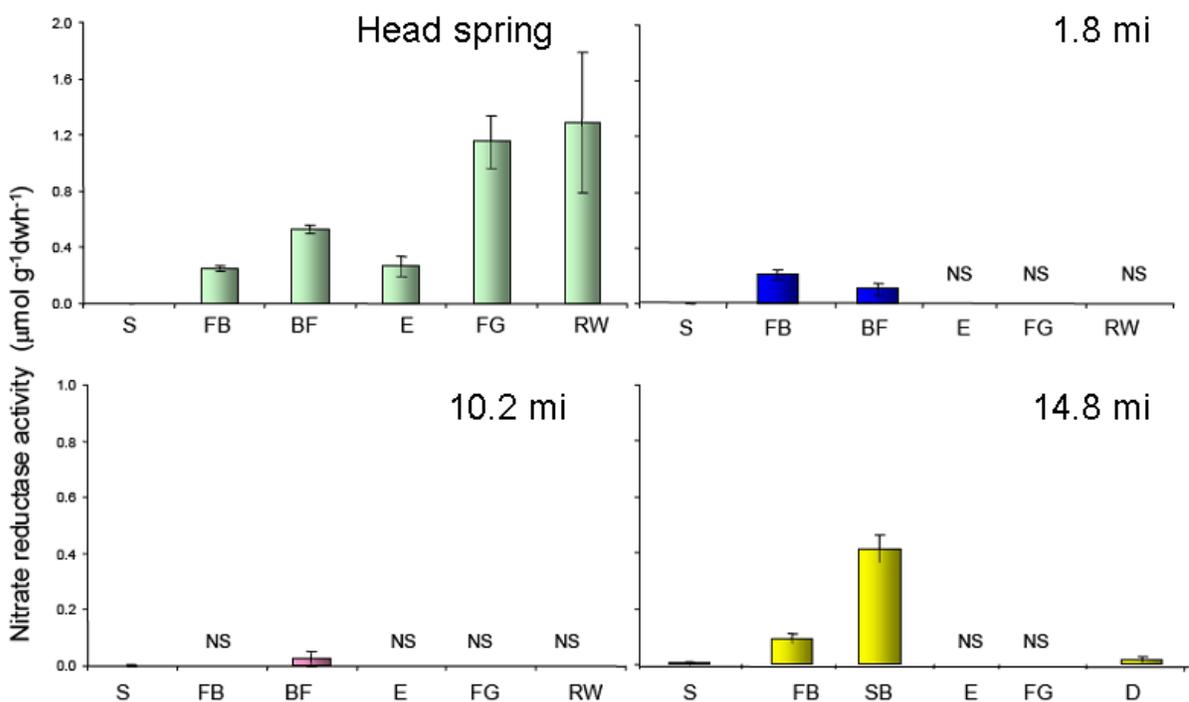


**Figure 5** – Idealized diagram of the nitrogen cycle occurring in a typical spring run system.

In springs it is unclear whether the abundance of N in the water column is the main source of N to the plant/epiphyte communities, or if SAV derive the majority of their N through root uptake. Several studies have noted that both pathways may be equally important for productivity in SAV (compare Madsen and Cedergreen 2002 and Xie *et al.* 2005). Both  $\text{NO}_3^-$  and  $\text{NH}_4^+$  are considered bioavailable; however,  $\text{NO}_3^-$  must first be reduced to  $\text{NH}_4^+$  before it can be assimilated into biomass. The enzyme, nitrate reductase is functional during this process, and thus, can be used to show active incorporation of  $\text{NO}_3^-$  in vegetation (Melzer and Kaiser 1986). At least one study has used this enzyme activity to assess potential  $\text{NO}_3^-$  use by biota in springs

with the findings that 1) higher water column  $\text{NO}_3^-$  levels resulted in higher levels of  $\text{NO}_3^-$  reductase activity and 2) enzyme expression in biofilms differed depending on substrate type (Inglett *et al.* 2007) (Figure 6).

In addition to inorganic N forms, it has also been noted that organic forms of N, such as amino acids and other small molecular weight N compounds (e.g., urea) can be a significant source of N to both microbial/algal communities (e.g., Berman and Bronk 2003; Linares and Sundback, 2006) and wetland macrophytes. This is important not only because of its role in nutrition, but also because in many aquatic systems, the presence of organic forms of N (in particular, urea) has been shown to effect algal species composition (e.g., Heil *et al.* 2007). Thus, both quality as well as quantity of N may be important factors determining the impact or fate of N in springs.



**Figure 6** – Nitrate reductase activity ( $\mu\text{mol NO}_2^-$  produced  $\text{g}^{-1}\text{dw h}^{-1}$ ) in sediment and algal biofilms from various substrates (S, sediment; FB, filamentous benthic algae; BF, brown filamentous; E, epiphytic algae; FG, floating green algae; RW, rock wall biofilm; D, detrital material; SB, submerged bark biofilm) at four locations along the Wekiva River. (from Inglett *et al.* 2007).

### Nitrogen mineralization

The biological transformation of organically combined nitrogen to ammonium nitrogen during the degradation of organic matter (ammonification) is one of the key mechanisms regulating the fate of nitrogen in aquatic systems. This process is strongly related to organic C decomposition, since a major portion of N in soil organic matter and plant detritus is bonded to C. As organic matter is decomposed, organic N is mineralized and released as ammonium. This process is achieved by a series of microbially-produced enzymatic processes (e.g. cellulose, cellulobioase,

glucosidase, aminopeptidase, protease etc.). Therefore, similar to C decomposition, the regulators of organic nitrogen mineralization include substrate quality, microbial biomass and enzyme activity, temperature, redox potential and hydrology. In a spring run, sites of ammonification would coexist with sites of organic matter/detrital matter accumulation or where organic matter has a rapid turnover as in the biofilms or the algal mats.

Depending on microbial N requirements, mineralized ammonium may be released, rapidly utilized by the microorganisms (immobilized), or taken up by plants. During decomposition, the C:N ratio reflects the mineralization-immobilization pattern of nitrogen, where a high C:N ratio promotes immobilization, while a lower ratio favors mineralization. During immobilization nitrogen is considered to be limiting to decomposer organisms. For this reason, initial N content of organic matter is a strong determinant of decomposition rates. Thus, organic matter with a low C:N ratio (such as algae or SAV) is considered labile, while those with high C:N ratios (such as wood, or senescent riparian vegetation) are considered more recalcitrant. Based on these factors, the production or consumption of  $\text{NH}_4^+$  in the water column in a spring can be highly variable.

Ammonium in the water column is derived from (1) decomposition of organic matter in the water column; (2) mineralization of organic nitrogen in the aerobic soil/sediment layer; (3) mineralization of organic nitrogen in the biofilms; and (4) diffusion and mass flow of ammonium from anaerobic soil layer to aerobic soil/sediment layer and water column. Saunders (2007) found that sediments in the Chassahowitzka River were reducing at depth, while Stevenson et al. (2007) measured porewater  $\text{NH}_4^+$  levels  $>10 \text{ mg L}^{-1}$  under mats of *Vaucheria* sp. Both studies indicate that sediments in a spring run system can act as a significant source of ammonium to the overlying aerobic soil layer and water column. Flux of ammonium is accomplished by diffusion, advection, bioturbation, and mixing at and near the soil-floodwater interface.

Once  $\text{NH}_4^+$  enters the water column, a variety of processes can affect its fate in a spring run/system. Ammonium is bioavailable, and therefore can be rapidly assimilated by phytoplankton, biofilm organisms, or SAV. Alternatively, if pH is high ( $>8$ ) a significant proportion of  $\text{NH}_4^+$  will be in the form of gaseous  $\text{NH}_3$  which can volatilize (flux out of the water column). Volatilization of  $\text{NH}_3$  can be significant in highly productive systems; however, in flowing waters with a high buffering capacity, it is unlikely that losses via this pathway are significant.

By far, the most likely fate of  $\text{NH}_4^+$  in the well-oxygenated water column of a spring run will be nitrification where microorganisms oxidize  $\text{NH}_4^+$  to  $\text{NO}_3^-$ . Autotrophic bacteria are dominantly responsible for the process of nitrification, and therefore these organisms do not require organic matter to function. Nitrifying populations of bacteria are likely to be found in all components of spring systems, and should be particularly concentrated at the aerobic/anaerobic interfaces where  $\text{NH}_4^+$  and  $\text{O}_2$  will both be available.

## **Denitrification**

Nitrate has a high oxidation potential (similar to oxygen) and can be used by a variety of organism groups as an electron acceptor (becoming chemically reduced). One of the most

significant and highly studied processes reducing nitrate is the process of denitrification where organisms couple organic C oxidation to nitrate reduction. This process is almost exclusively anaerobic (a few aerobic examples have been found). Many of the organisms capable of denitrification are facultative and are capable of surviving in transitional (moderately anaerobic) or fluctuating aerobic/anaerobic conditions. In fact, in many aquatic systems denitrification zones occur in close proximity to the aerobic zones of  $\text{NO}_3^-$  production (nitrification).

Denitrifying organisms are predominantly heterotrophic. Therefore high organic matter C deposits (sediments, debris dams, biofilms, and algal mats) are the likely zones for occurrence of denitrification in spring systems. Alternatively, turbulent flow in many springs would likely be sufficient to advect significant quantities of water column  $\text{NO}_3^-$  into anaerobic sediments in the hyporheic zone (Triska *et al.* 1993; Duff *et al.* 1998). Hyporheic zones include regions beneath the stream channel and regions adjacent to the channel where stream water is exchanging with interstitial waters at rates and scales larger than those limited by diffusion. The majority of springs are too well oxygenated for denitrification to proceed at any great rate in the water column; however, dense SAV or heavy biofilm growths possess enough C and can sufficiently reduce water flow to induce anaerobic conditions either spatially (e.g., in microsites) and/or temporally (short-term transient events or diel cycles). This would be particularly important at night in the absence of photosynthesis.

To date, the only reported measurements of denitrification potential in Florida springs have been conducted in Wekiwa Springs/Wekiva River by Inglett *et al.* (2007). In that study, potential denitrification rates were measured in sediments and algal biofilms at four sites ranging from the headspring to 14.8 miles downstream (Figure 7). Observed rates ranged from 10  $\text{nmol N}_2\text{O produced gdw}^{-1} \text{ h}^{-1}$  in surface sediments (0-10 cm) to 170  $\text{nmol N}_2\text{O produced g}^{-1}\text{dw h}^{-1}$  in biofilms collected from bark samples from the headspring. The rates measured in the Wekiwa/Wekiva system are similar to DEA rates reported in wetlands (0.5 to 20  $\text{mg N}_2\text{O kg}^{-1} \text{ soil h}^{-1}$ ; White and Reddy 2003), estuarine sediments (15-130  $\text{nmol N cm}^{-3} \text{ d}^{-1}$ ; Joye *et al.* 1996), and in periphyton mats (1.9  $\mu\text{mol N}_2\text{O m}^{-2} \text{ h}^{-1}$ ; Triska and Oremland, 1981). Based on their results, Inglett *et al.* (2007) concluded that despite a conclusive trend in DEA downstream (along a depletion gradient of surface water  $\text{NO}_3^-$ ) denitrification appeared to be a significant pathway for N removal from the system.

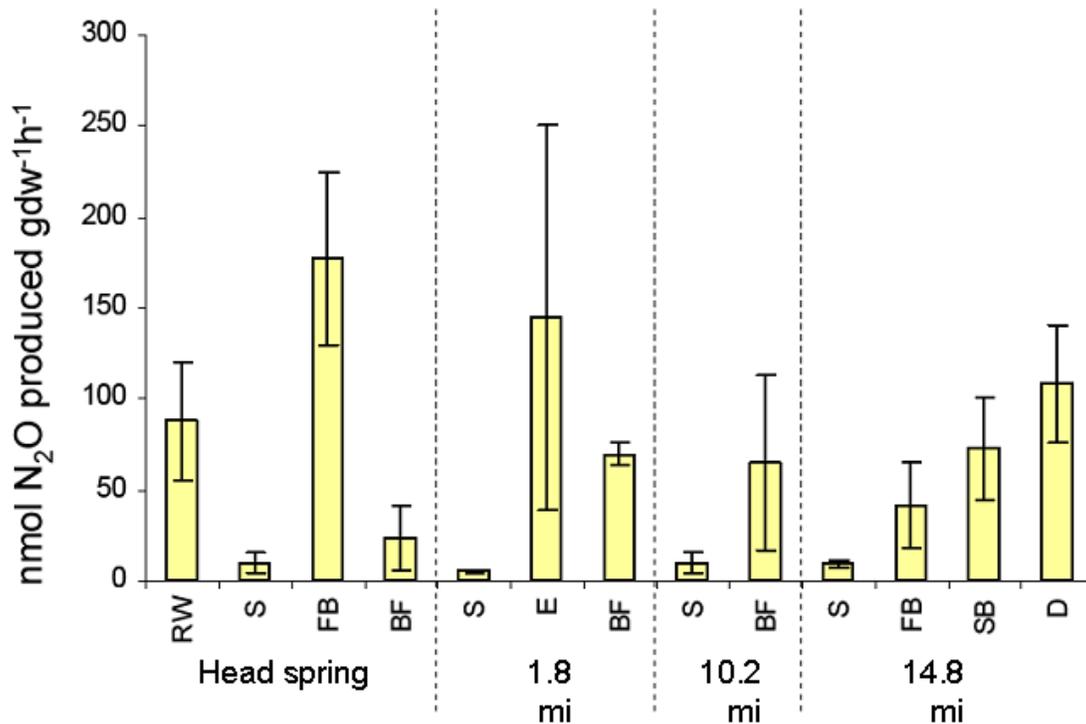
### **Alternative N pathways**

Conversion of  $\text{NO}_3^-$  can occur by additional pathways. Some of these have been identified for a number of years while others have emerged more recently (reviewed by Burgin and Hamilton 2007) (Figure 8). Many of these pathways have yet to be identified in springs or streams; however, the potential exists for these processes to account for significant reduction of  $\text{NO}_3^-$  in aquatic systems. For this reason, we will discuss them briefly here.

The high energetic potential of using  $\text{NO}_3^-$  as an electron acceptor is not solely possessed by heterotrophic bacteria. Several pathways have also been identified that can reduce  $\text{NO}_3^-$  through the oxidation of other reduced compounds. Identification of these new mechanisms has challenged the classical view of denitrification in aquatic systems. Among these,  $\text{NO}_3^-$  reduction can be coupled to the oxidation of either reduced iron (Fe(II)) or sulfur ( $\text{S}^0$ ,  $\text{S}^{2-}$ ). The importance

of these lithotrophic pathways to overall rates of denitrification has not yet been determined. Likewise, in springs and lotic systems, Fe(II) and S<sup>2-</sup>-based denitrification has received little attention. It is not unlikely that these pathways could be significant given the presence of iron and sulfur in many spring systems and the myriad of reactions occurring at the oxic/anoxic interfaces of microbial mats, biofilms, or sediments.

Another pathway for NO<sub>3</sub><sup>-</sup> reduction can convert NO<sub>3</sub><sup>-</sup> into NH<sub>4</sub><sup>+</sup>. This process, called dissimilatory nitrate reduction to ammonium (DNRA), is different from the assimilatory process in that NH<sub>4</sub><sup>+</sup> produced is not directly incorporated into biomass. Fermentative DNRA couples organic matter oxidation to NO<sub>3</sub><sup>-</sup> reduction by fermentation reactions (Tiedje 1988).

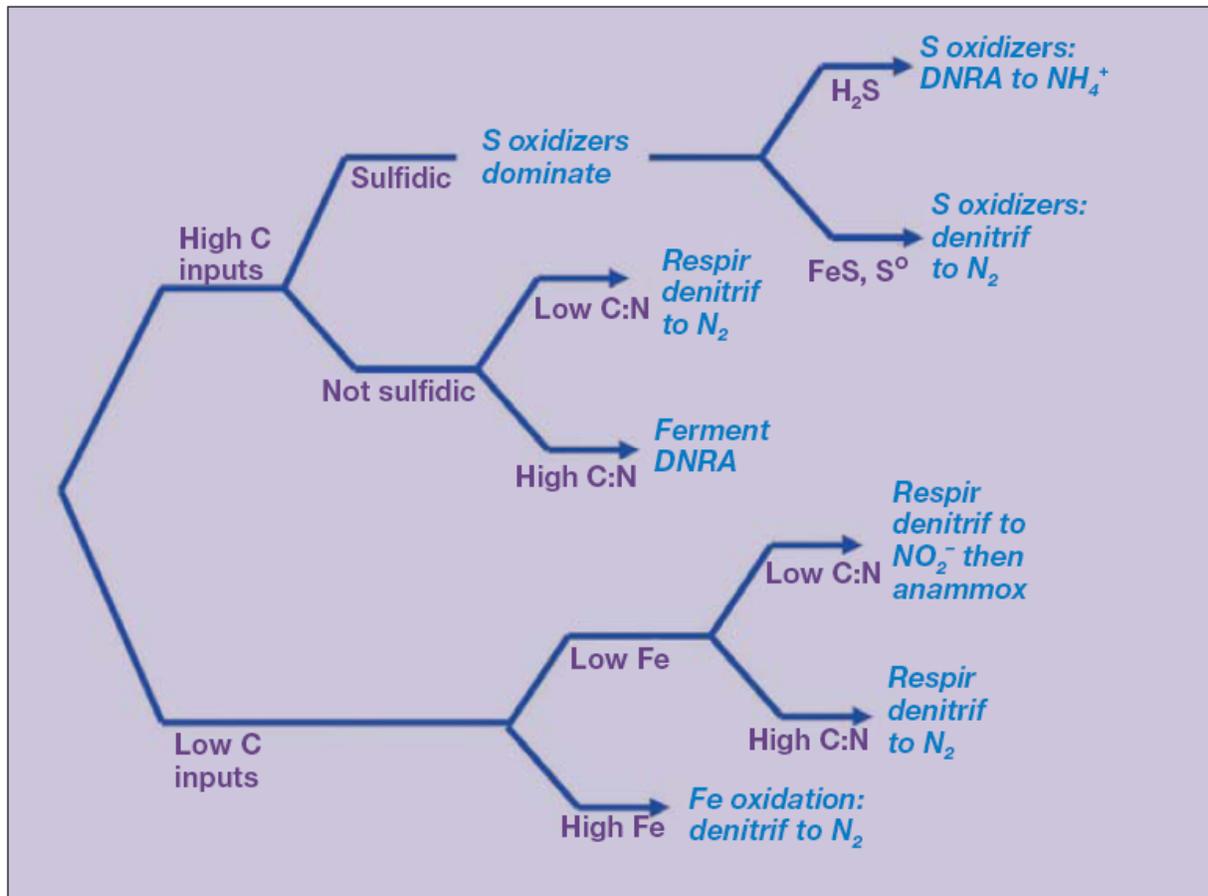


**Figure 7** – Denitrifying potential in sediments, and algal biofilms collected from various substrata. S, sediment; FB, filamentous benthic algae; BF, Brown filamentous algae; E, epiphytic biofilms; RW, Biofilms attached to rock wall; D, detrital material; SB, algal biofilm on submerged bark. (From Inglett et al. 2007)

Another form of DNRA is chemolithautotrophic and couples NO<sub>3</sub><sup>-</sup> reduction to oxidation of reduced S (e.g., S<sup>2-</sup>). Strongly reducing conditions with high contents of organic matter or high concentrations of reduced Fe and S are required for DNRA. As stated for chemotrophic denitrification, conditions may exist for DNRA activity in springs; however, these areas would be highly localized to accumulations of organic matter and/or slow or no flow conditions.

Most recently, a novel pathway for N conversion to N<sub>2</sub> has been identified which adds even more uncertainty to the aquatic N cycle. The process known as anaerobic ammonium oxidation (Anammox) has now been identified in a number of freshwater systems including lakes and

wetlands. This process occurs in strictly anaerobic conditions, and couples the reduction of  $\text{NO}_2^-$  with the oxidation of  $\text{NH}_4^+$  to produce  $\text{N}_2$  gas. Anammox bacteria appear to be autotrophic, and the presence of organic matter slows the anammox process. Another important requirement for the anammox process is the presence of  $\text{NO}_2^-$  which is rarely observed in most systems.



**Figure 8** – Hypothesized controls on predominant dissimilatory pathways of  $\text{NO}_3^-$  removal. C inputs refer to labile organic carbon available to microbes. Sulfidic refers to the presence of either free sulfide ( $\text{S}^{2-}$ ), elemental S ( $\text{S}^0$ ). Of these S forms, only free sulfide inhibits denitrification and thus promotes DNRA. C:N ratios refer to the ratio of labile organic carbon to nitrate. Respir = respiratory; denitrif = denitrification; DNRA = dissimilatory nitrate reduction to ammonium; ferment = fermentative. (From Burgin and Hamilton 2007).

Nitrite accumulation is possible however, and several conditions of spring systems may be adequate for  $\text{NO}_2^-$  accumulation to occur. The formation of  $\text{NO}_2^-$  is largely a byproduct of incomplete denitrification or partial ammonium oxidation. For  $\text{NO}_2^-$  accumulation to occur in the denitrification and DNRA processes,  $\text{NO}_3^-$  reduction must occur at a greater rate than that of  $\text{NO}_2^-$  reduction. One way in which incomplete denitrification can occur is the lack of suitable organic substrates, or high concentrations of  $\text{NO}_3^-$ . Similarly, in the nitrification pathway, elevation of  $\text{NO}_2^-$  concentrations can occur only if the oxidation of  $\text{NH}_4^+$  is greater than that of  $\text{NO}_2^-$ . Both of these scenarios have been reported to occur in the aquatic environment (Herbert 1982; Smith *et al.* 1997). *Nitrobacter* spp., which are responsible for the oxidation of  $\text{NO}_2^-$  in

the nitrification process, may be partially inhibited by free ammonia ( $\text{NH}_3$ ) (Anthonsieu et al. 1976; Smith *et al.* 1997), leading to  $\text{NO}_2^-$  accumulation. Likewise,  $\text{NO}_2^-$  accumulation is a common trait of DNRA (Cole 1988) resulting from either inhibitory effects of  $\text{NO}_3^-$  on  $\text{NO}_2^-$  reductase (Smith, 1982) or repression of this enzyme (Paul and Beauchamp 1989).

Higher than expected  $\text{NO}_2^-$  levels ( $100\text{-}200 \mu\text{g N l}^{-1}$ ) have been reported in some European rivers, particularly in watersheds receiving large inputs of nitrogenous pollution (Smith *et al.* 1995; Kelso *et al.* 1997). In one study, Kelso *et al.* (1997) found that  $\text{NO}_2^-$  accumulation in six Irish rivers corresponded with  $\text{NO}_3^-$  declines via DNRA activity. This process was occurring in areas with high organic matter contents (Kelso *et al.* 1999), leading these authors to conclude that high  $\text{NO}_3^-$  concentrations, free ammonia, or high flow velocity created conditions suitable for  $\text{NO}_2^-$  accumulation to occur. All of these studies suggest  $\text{NO}_2^-$  may be much more abundant than previously thought. Therefore, it is likely that  $\text{NO}_2^-$  levels may be sufficient to support alternative N cycling pathways (such as anammox) in more aquatic ecosystems than previously thought.

### **Overall N Cycle**

It is uncertain what role spring systems play as sinks, sources, or transformers of N. Several studies have reported declining inorganic N concentrations (primarily  $\text{NO}_3^-$ ) with distance down spring run/river (e.g., DeBrabandere *et al.* 2007; Inglett *et al.* 2007; Mytyk and Delfino 2004) (Figure 7). In general lotic systems should result in a transformation of N, including conversion of DIN to DON through biotic uptake and senescence. Increases of DON with distance through a run have also been observed (Inglett *et al.*, 2007; Wetland Solutions, Inc., 2006). The study by Wetland Solutions, Inc. (2006) also indicates that DON production in spring runs is likely the result of excess N, where Wekiva and Rock Springs Run (both  $\text{NO}_3^-$ -impacted) show much higher production of DON in specific river segments, while Alexander and Juniper spring runs (reference conditions) show smaller production (or even net reduction) of DON.

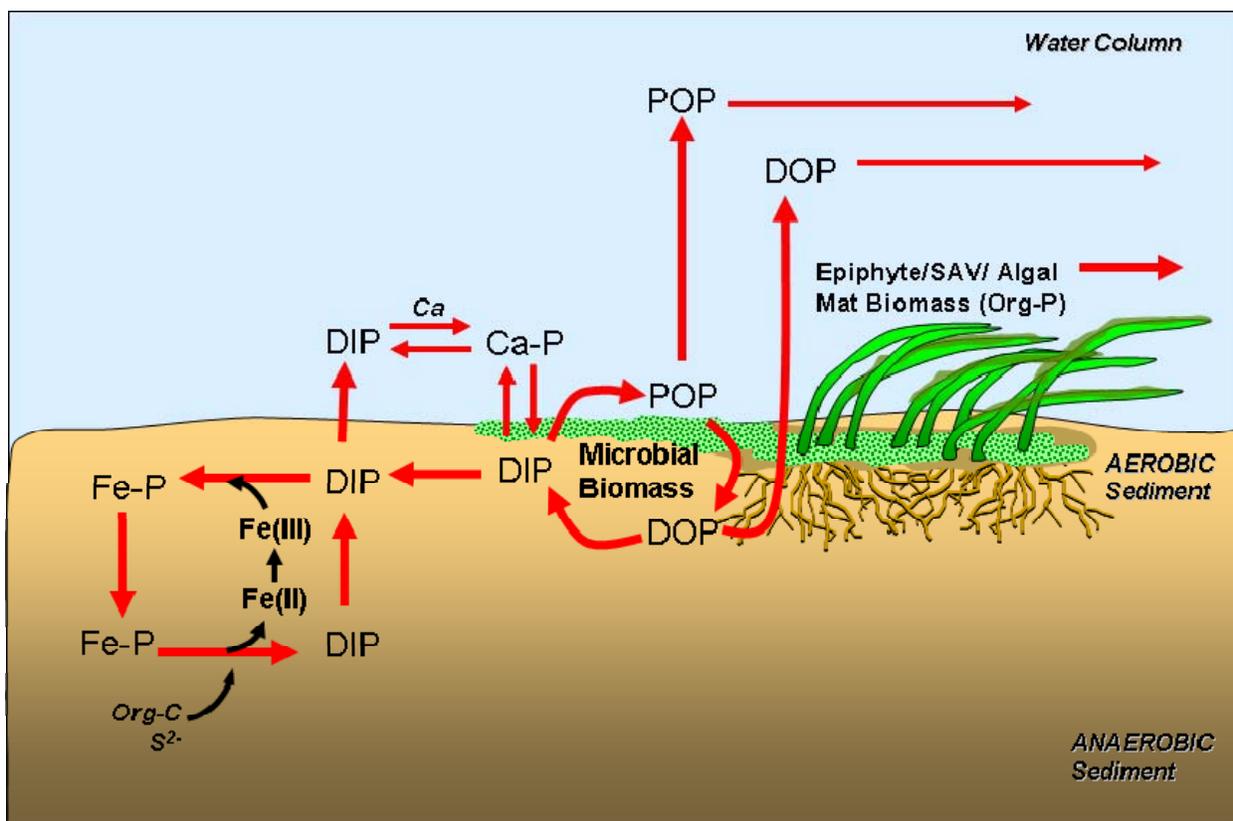
It is also quite likely that springs are active zones of denitrification. Evidence of denitrification in springs comes from at least one study (Inglett *et al.* 2007), and the conditions exist for other unexplored areas where denitrification could occur (Saunders 2007). Areas with direct inputs of reduced groundwater into sediments with low organic matter content, or water column  $\text{NO}_3^-$  being advected into anaerobic sediments, are also key conditions for alternative denitrification pathways to occur in springs (Grimaldi and Chaplot 2000). In systems with significant quantities of iron or sulfur, additional lithotrophic N loss mechanisms may also be operating. Nitrogen cycle studies and budgets of springs are not available at this time. For this reason it is difficult to attribute the several examples of declining  $\text{NO}_3^-$  to either the process of biotic uptake or denitrification; however, this question is of critical importance for management of springs as nutrient sources.

### **Phosphorus**

Phosphorus (P) is one of the major nutrients limiting productivity in freshwater ecosystems. During the last five decades, many studies have been conducted to determine the fate of P in freshwater systems, including springs, streams and other aquatic ecosystems. At the landscape

level, streams and wetlands form a critical interface between uplands and adjacent water bodies, as all of these ecosystems are hydrologically linked. Water and associated nutrients (such as P) are transported from uplands either by subsurface or surface flow. An idealized P cycle for spring systems is shown in Figure 9.

Both biotic and abiotic mechanisms regulate relative pool sizes and transformations of P compounds within the water column and sediment. Alterations in these fractions can occur during flow in streams and wetlands that depend on the physical, chemical, and biological characteristics of the systems (Meyer and Likens 1979). Thus, when evaluating P fate and transport in springs/spring runs, both biotic and abiotic processes must be considered. Biotic processes include assimilation by vegetation, biofilm/periphyton and sediment microorganisms. Abiotic processes include sedimentation, adsorption by sediments and organic/inorganic substrates, precipitation, and exchange processes between sediment and the overlying water column.



**Figure 9** – Idealized diagram of the phosphorus cycle occurring in a typical spring run system. PIP=particulate inorganic P, POP=particulate organic P, DIP=dissolved inorganic P, DOP=dissolved organic P, Fe-P=iron bound P, Ca-P=calcium bound P.

### Forms of phosphorus in water column and sediments

Phosphorus entering a spring system is typically present in both organic and inorganic forms. The relative proportion of each form depends upon the geology, soil, vegetation and land use

characteristics of the aquifer, or springshed. To trace the transport and transformations of P, it is convenient to classify forms of P entering these systems as (1) dissolved inorganic P (DIP); (2) dissolved organic P (DOP); (3) particulate inorganic P (PIP), and (4) particulate organic P (POP) (Figure. 9). The particulate and soluble organic fractions may be further separated into labile and refractory components. Dissolved inorganic P is considered bioavailable, whereas organic and particulate P forms generally must undergo transformations to inorganic forms before being considered bioavailable.

Inorganic P forms in sediments are characterized based on their differential solubilities in various chemical extractants. The fractionation schemes the includes following pools: (1) exchangeable P; (2) Fe and Al-P or non-apatite P; (3) Ca- and Mg-bound P or apatite P; and (4) residual P or nonreactive P (Hieltjes and Lijklema 1980; van Eck 1982; Psenner *et al.* 1988; Cooke *et al.* 1992; Olila *et al.* 1995; Ruttenberg 1992; Reddy *et al.* 1998). The example in Figure 10 shows labile and non-labile pools of P in selected stream sediments of south Florida (Reddy *et al.* 1995a). In this example, the inorganic P extracted with neutral salt such as KCl represents loosely absorbed P, which usually accounts for less than 2% of the total P. This pool of P is considered bioavailable as it is sufficiently labile to enter porewater for uptake of plants or potential flux into the overlying water column.

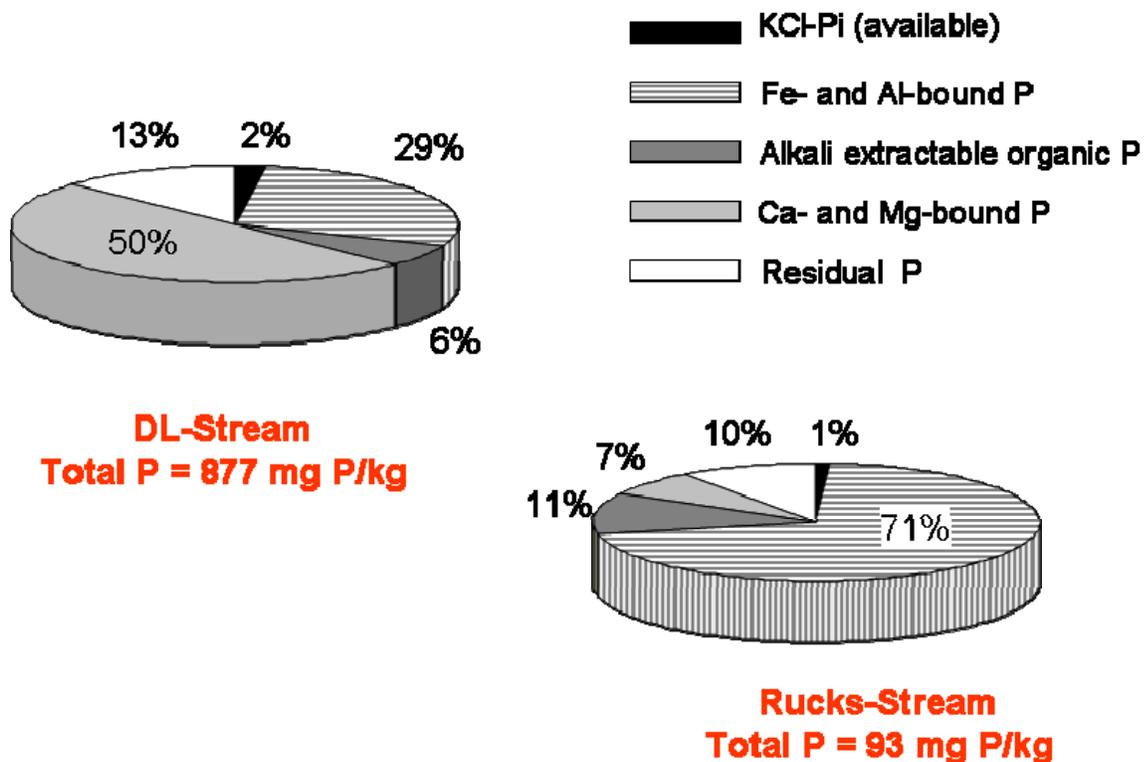
The NaOH-P<sub>i</sub> represents inorganic P associated with Fe and Al, and represents P not readily available. The HCl-P<sub>i</sub> represents P associated with Ca/Mg, is also relatively stable and not readily available. Alkali extractable organic P includes both readily available organic P (microbial biomass P) and slowly available organic P (P associated with fulvic and humic acids). Residual P represents highly resistant organic P or unavailable mineral bound P not extracted either with alkali or acid. The Ca-bound P such as apatite has been found to be unavailable (Pettersson 1986; Gunatilaka 1988), while the redox-sensitive Fe-bound P may become available under anaerobic conditions (Figure 9) (Wildung *et al.* 1977; Furumai and Ohgaki 1982; Hosomi *et al.* 1981).

Although these categories allow some evaluation of properties, mobility, and quantitation, the extent of bioavailability among each of the organic P fractions can be obscure and variable. Bioassays with microbes (bacteria, algae) can yield insights on instantaneous bioavailability, but clearly sorption and desorption reactions can shift the solubilities and availabilities rapidly. These exchange reactions are markedly influenced by microbially-induced redox shifts and hence can change rapidly in water and especially in sediments.

Organic P forms may dominate the water column, with the majority of P occurring as particulates in biomass of algae and macrophytes. Some of the organic P may be associated with dissolved organic phases. The relative proportion of organic P in sediments of springs, spring runs and streams may be smaller as compared to riparian zones. In many streams accumulation of organic matter may be lower compared with riparian zones, thus resulting in lower accumulation of organic P. In organic matter, P is a major constituent, as nucleic acids (DNA and RNA), in phospholipids of membranes, and as monoesters of a variety of compounds, particularly those involved in biochemical pathways. In microorganisms and algae, more than half of the organic P is in nucleic acids, whereas phospholipids and monoesters constitute the remainder in varying proportions (Magid *et al.* 1996). Many organisms can store orthophosphate

or polyphosphates (Cembella *et al.* 1983, 1984). In plants, inositol hexaphosphate (IHP) can form a major storage compound for P, particularly in seeds.

The epiphytic community living on submerged surfaces acquires P from both the water column and from the supporting ‘host’ substrate. Dissolution of inorganic substrates such as minerals can potentially provide some essential nutrients to algae and microbes colonizing on them. Although relatively little of the total P pool within actively growing macrophytes is released, this released P can be important to certain epiphytic species that grow adnate to the macrophyte tissue (Moeller *et al.* 1988). Even when P concentration in the overlying water is high, some nutrients are obtained from the macrophyte simply because diffusion within the complex epiphytic community is too slow to meet demands (Wetzel 1993a). The periphyton, rather than the macrophytes, functions as the primary scavenger for limiting nutrients such as P from the water column.



**Figure 10** – Fractionation of sediment phosphorus in South Florida streams dominated by calcium (DL Stream) and iron (Rucks Stream). (From Reddy and DeLaune 2008).

### Mineralization of organic phosphorus

Active cycling of organic P in sediments and water column is largely mediated by microbial metabolism. In order to understand cycling of organic P it is essential to evaluate the organically-bound P within compounds of the microbial biomass and the organismal dynamics, particularly the bacteria, as they influence organic P dynamics. Microbial metabolism influences rates of mineralization of organic P in two primary ways: (1) by direct biochemical mobilization using extracellular or periplasmic hydrolases, particularly from inositol phosphates, and (2)

through alteration of ionic composition, redox, pH, and other conditions that in turn can alter the efficacy of P binding to particulate organic matter, clays, and other soil particles, as well as solubility of organic P and its potential enzymatic hydrolysis (Reddy *et al.* 2005).

The availability of phosphate monoesters requires enzymatic cleavage of the ester linkage joining the inorganic P group to the organic moiety. Such hydrolysis is achieved by phosphomonoesterases (phosphatases) bound to or within the cell membrane or by dissolved enzymes released extracellularly within the interstitial solution adjacent to the cells. There is a general lack of specificity for the organic moiety that include, beyond phosphate monoesters, substrates such as diesters and phosphoanhydrides such as pyrophosphates, ADP, and ATP. The phosphatases of bacteria, algae, fungi, and higher plants are constitutive inorganic P-irrepressible enzymes, which tend to be intracellular and function in intermediary metabolism, or are phosphate ester-induced inorganic P-repressible enzymes, which are usually membrane-bound and function in extracellular P cleavage (Cembella *et al.* 1983).

Bacteria and microalgae growing on soil particles and within interstitial water of sediments of aquatic ecosystems are able to utilize exogenous organic P compounds through enzymatic hydrolysis of terminal phosphate groups. A broad spectrum of dissolved organic P compounds can sustain growth of these organisms, including glycerophosphate, D-glucose 6-phosphate (G-6-P), adenosine 5'-monophosphate (AMP), cytidine 5'-monophosphate (CMP), guanosine 5'-monophosphate (GP), adenylic acids, and phosphonate compounds (Cembella *et al.* 1983, 1984). Because of the high reactivity of solubilized P, the distance between sites of enzymatic hydrolysis of organic P and sites of uptake must be short, often in immediate juxtaposition (Tarafdar and Jungk 1987). Inactivation by reaction with the soil matrix increases with time after release and with greater available surface areas associated with decreasing size of soil aggregates (Linquist *et al.* 1997a).

Expectedly, a positive correlation has been found between bacterial biomass and phosphatase activity in surficial lake sediments (Reichardt 1978). Correlations between enzyme activities and bacterial biomass were less robust several centimeters below the sediment-water interface. A higher affinity of phosphatases was found in sediments where a low mobility of sediment P occurred (Pettersson unpublished; cited in Boström *et al.* 1982). The lowest substrate affinity (highest half-saturation constants) was found in organic sediments with low chemical adsorption capacities that release P under anaerobic conditions. Low availability of P in sediments often induces production of phosphatase activity with high affinity for substrates.

### **Biotic and Abiotic Ca Interactions**

In calcium dominated streams, the presence of high  $\text{Ca}^{2+}$  can result in formation of complex calcium phosphate compounds of varying solubilities such as calcium phosphate, dicalcium phosphate, beta-tricalcium phosphate, octacalcium phosphate, and hydroxyapatite. Under these conditions, the phosphorus concentration in the pore water of calcareous sediments is a function of  $\text{Ca}^{2+}$  activity. Solubility of these compounds decreases with an increase in Ca content. Insoluble beta-tricalcium phosphate is more likely to be found at a high pH. Thermodynamically, apatite is the most stable compound, while at relatively high phosphate concentrations, dicalcium phosphate or octacalcium phosphate may form and slowly transform to the more stable phase,

hydroxyapatite. These precipitation reactions can occur on surfaces of calcite. The amount of exposed surface will determine the amount of phosphorus precipitated. In Ca-saturated clay, calcium phosphate will precipitate as a separate phase above pH 6.5; however, this depends on the concentration of phosphate ions in sediment pore water.

Algal communities can play a major role in regulating P concentrations of the water column. Algae can assimilate both organic and inorganic forms of P (Bentzen *et al.* 1992), and can induce marked changes in pH and dissolved oxygen concentration of water column and soil-floodwater interface (Carlton and Wetzel 1988). On a diurnal basis these processes can increase pH to as high as 10, depending upon the buffering capacity of the water column. These changes can potentially influence the solubility of P, especially in streams. Some studies (e.g., Hartley *et al.* 1997) have demonstrated that this process can result in significant co-precipitation of phosphorus with calcite in the presence of photosynthesizing algae, like *Chlorococcum* sp.

Retention of inorganic phosphorus by precipitation will be significant in spring waters with high  $\text{Ca}^{2+}$  and alkalinity. In calcium-dominated streams (e.g., chalk streams) a significant portion of water column phosphorus can co-precipitate with  $\text{CaCO}_3$  where dissolved  $\text{Ca}^{2+}$  is available. House (1990) attributed only 6% of the overall phosphorus removal to co-precipitation, while the remainder was due to biological uptake. However, Salinger *et al.* (1993) found metastable calcium phosphate species to be a predominant form of phosphorus transported in the River Jordan. Several studies showed inhibition of calcite growth in the presence of high phosphorus concentrations (Avnimelech 1980; Dove and Hochella 1993) while at low phosphorus concentrations, coprecipitation of phosphorus with calcite has been observed (House and Donaldson 1986; Giannimaras and Koutsoukos 1987; Kleiner 1988). Approximately 75-90% of the phosphorus precipitated was solubilized when pH levels decreased to below 8 as a result of an increase in carbon dioxide levels (Diaz *et al.* 1994).

### **Biotic and Abiotic Fe Interactions**

In spring and stream sediments dominated by iron minerals, reduction of ferric (Fe(III)) hydroxides and complexes results in ferrous Fe (Fe(II)), and adsorbed phosphate to be mobilized into soil pore water (Figure 9). Such conditions are of particular importance in areas where organic matter (and reducing conditions) can be transient, or when ground water varies temporally between aerobic and anaerobic states. Under strongly reducing conditions where sulfate is reduced to hydrogen sulfide, some  $\text{S}^{2-}$  can react with iron compounds (both Fe(III) and Fe(II)) resulting in the precipitation of Fe-S compounds. In this manner, sulfide formation coupled to sulfate reduction can displace iron-bound P to enhance the release of P into the interstitial waters (Wetzel 2001). The relative P release from sediments can be significantly higher as sulfate concentrations increase from natural or anthropogenic sources, particularly in oligotrophic, softwater lakes and rivers. In highly reduced soils with low sulfate content, microbial reduction generates mostly Fe(II) compounds from microbial reduction of Fe(III) oxide.  $\text{PO}_4^{-3}$  can be retained with Fe(II) compounds, but Fe-associated  $\text{PO}_4^{-3}$  is released when amorphous Fe(III) oxide is converted to iron sulfides (Roden and Edmonds 1997). Thus, the conversion of reactive soil Fe compounds to iron sulfides by sulfate-reducing bacteria can lead to a more efficient release of Fe-associated  $\text{PO}_4^{-3}$  than does direct microbial Fe(III) oxide reduction.

## Overall P Cycle

Springs with high N availability should respond with higher rates of P uptake and subsequent release of DOP following senescence of biomass. Many springs do show such an increase of DOP with distance from the boil (e.g., Wekiva; Inglett *et al.* 2007). In these cases, springs should act as transformers of P or act as a small to moderate sink. In other cases, springs may actually serve as sources of P. This could occur through accumulation and mineralization of riparian vegetation deposited in the spring run, or solubilization of P contained in sediments emerging at the boil.

The process of P exchanges across the sediment-water interface is regulated by mechanisms associated with mineral-water equilibria, sorption processes (particularly ion exchange), oxygen and other electron acceptor-dependent redox interactions, and the physiological and behavioral activities of many biota from bacteria, algae to other living communities. Exchange rates between various deposits of P and the interstitial water of the sediments depend on local adsorption and diffusion coefficients and their alteration by enzyme-mediated reactions of the microbiota. The sink/source function of springs could be transient, where there are periods of storage/transformation followed by periods of flux/release. For this reason, whether individual springs act as sinks, sources, or transformers of P is highly dependent on the spring, and its physical, chemical, and biological characteristics.

## Sulfur

The idealized sulfur cycle for spring systems is shown in Figure 11. The predominant forms of sulfur are organic-S (existing mostly as protein/AA-S), sulfide ( $S^{2-}$ ), and sulfate ( $SO_4^{2-}$ ).

### Sulfide Oxidation

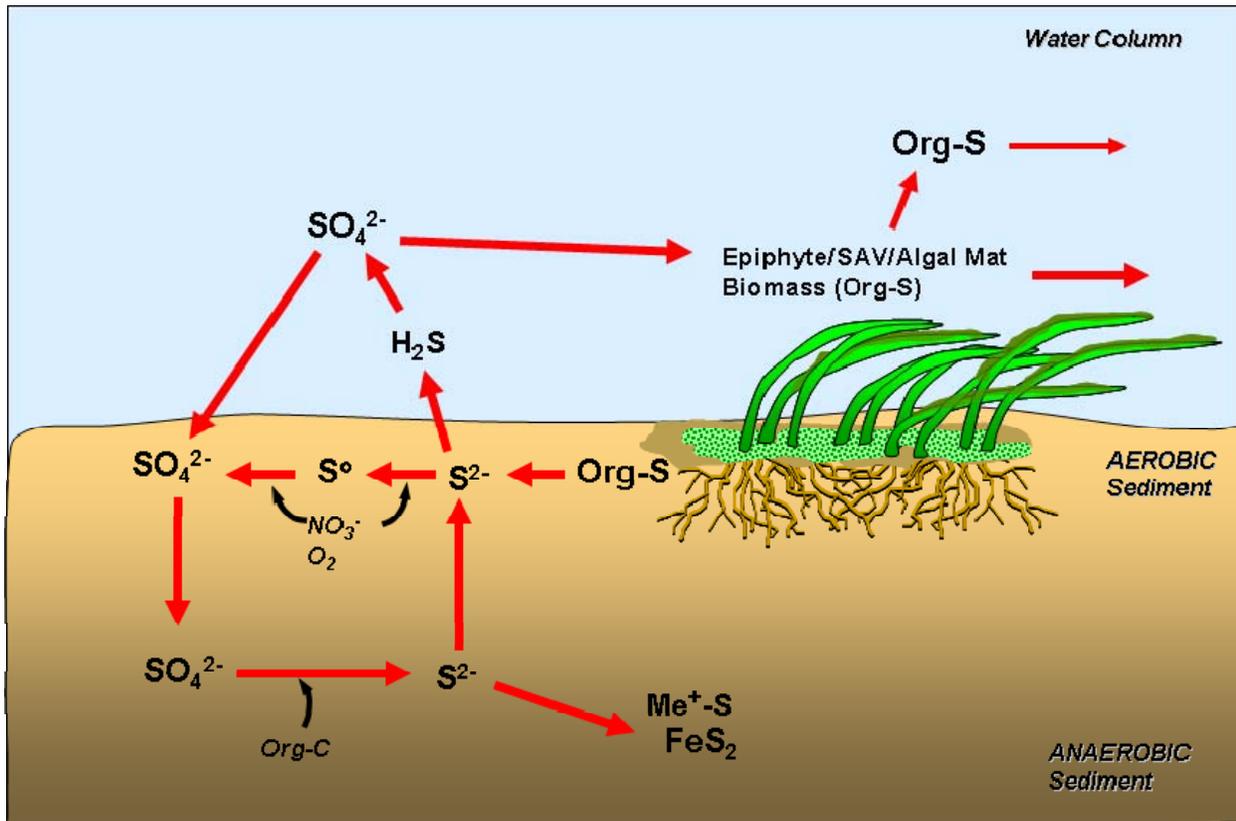
Emerging groundwater potentially contains quantities of  $S^{2-}$  that become oxidized when contacting atmospheric and photosynthetically-produced  $O_2$  near the boil/vent. This reaction is thermodynamically favorable, and several groups of bacteria (e.g., *Thiobacillus* spp.) can utilize this energy yield to fix atmospheric C (lithoautotrophy), or respire organic C (heterotrophy). Data from several springs indicate this process may be occurring. Sulfide is rarely determined in routine monitoring, but some springs show post-boil increases in  $SO_4^{2-}$  (Wekiva, e.g.; Wetlands Solutions, Inc. (2005)) indicating that  $S^{2-}$  oxidation could be occurring.

Springs with high  $SO_4^{2-}$  content may also host this process, but it likely occurs pre-boil (e.g., Juniper Springs). Other evidence for sulfur oxidizing pathways in spring systems comes from a study by Caldwell *et al.* (1975) who found large numbers of S-oxidizing bacteria including the genera *Thiothrix* and *Thiocystis* in a sulfur spring in the Florida Keys (Flowing Well) and several springs associated with lakes in Michigan. These bacteria were found attached as filaments or mucilage-encased microcolonies on aerobic surfaces of plants, interspersed with other filamentous algae, or on decomposing organic matter. Gradient culture techniques revealed that these S-oxidizing genera were culturable even at the lowest S levels tested ( $55 \mu\text{mol S l}^{-1}$ ).

## Sulfate Reduction

Sulfate present in the water column of spring runs can be utilized as an electron acceptor by select bacteria decomposing simple organic substrates (dissimilatory sulfate reduction). This process where  $\text{SO}_4^{2-}$  becomes reduced to  $\text{S}^{2-}$  only occurs under highly reducing conditions with redox potentials less than 200 mV. For this reason, measurable sulfate reduction is likely only possible in slow-moving spring runs or areas with high organic matter sediments (algal mats, SAV beds, litter accumulations).

Sulfate reducers are obligate anaerobes that couple oxidation of organic substrates to carbon dioxide with the reduction of the terminal electron acceptor sulfate to sulfides (Widdel 1998). Sulfate reducing bacteria cannot synthesize hydrolytic enzymes, thus they cannot hydrolyze polymers such as polysaccharides, and many groups cannot use monomers such as monosaccharides (e.g., glucose) as substrates for energy. As a result, sulfate reducers are dependent on fermenting bacteria to oxidize monomers to simple readily utilizable organic compounds.



*Figure 11 – Idealized diagram of the sulfur cycle occurring in a typical spring run system.*

## Overall S Cycle

Because of the prevalence of oxidizing conditions in the water column, most spring runs should result in a net export of oxidized S species ( $S^{2-}$  oxidized post boil). With the exception of the initial degassing at the boil of some high S springs, most Florida springs should not emit significant amounts of S as  $H_2S$ . The only other significant quantity of S compounds should be through the transport (loss) of organic-S in organic matter carried downstream.

## EFFECTS OF INCREASED NUTRIENTS

### Carbon/Organic Matter Impacts

Common ecological theory would predict that addition of nutrients to a nutrient-limited system will enhance productivity and lead to greater overall cycling of C through the organic pool. However, a study of four spring runs by Wetland Solutions, Inc. (2005) found that spring runs with higher nutrient levels (Wekiva and Rock Springs) had lower ecosystem productivity estimated through total system metabolism (DO gain/loss) than the reference spring runs (Alexander and Juniper). Increased nutrients leading to algal dominance and growth (even if not resulting in an overall increased productivity) would still reflect a change in the quality of organic matter in the system and could have cascading effects through other heterotrophic communities both locally and further downstream.

Other aspects of the C cycle in springs could be altered through the addition of C. Increased C inputs could occur through increased sewage/pollutant discharges which would act to raise the concentration of solids C or DOC. Management of exotics/invasives could also have profound impacts on nutrient dynamics through increased and episodic deposition of C following herbicide application. The effects of large discharges of C into streams are well known and most likely the same effects apply to springs, spring runs and springsheds. In particular, the introduction of large C quantities increase respiration losses of oxygen and other electron acceptors and increase production of significant quantities of reduced compounds potentially affecting many of the nutrient cycles, including N (increased denitrification), or P (increased release of iron-bound P).

### Nitrogen Impacts

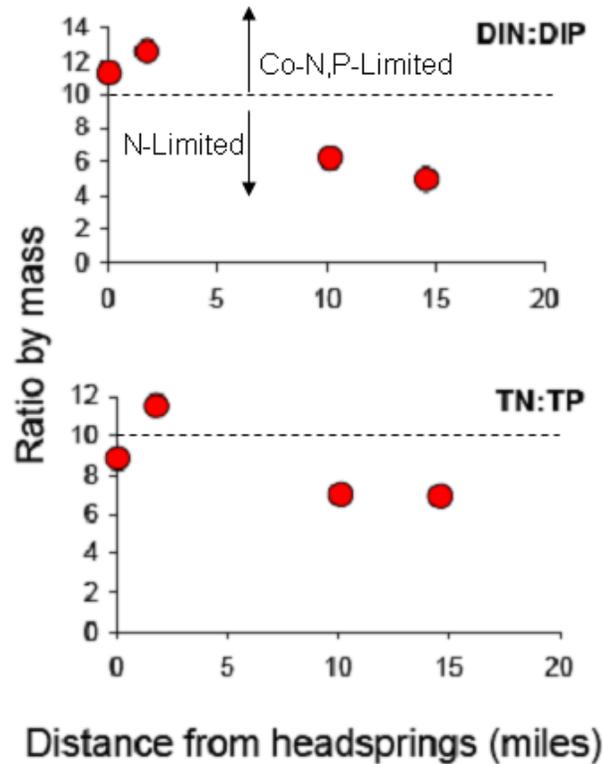
In the natural state, most Florida springs contain high levels of inorganic P, and based on N:P ratios, are N-limited. In the N-limited state, springs would be considered highly susceptible to N pollution. One of the key responses to N in these cases would be increased biomass or shifts in dominant species of all groups, including algae, macrophytes, fungi and bacteria. One example of this would be that under N-limiting conditions there should be a dominance of particular N-fixing microbiota. With the shifts in species composition, changes in C abundance and quality can also be expected. For example, increased algal production would result in lower amounts of structural plant C compounds (cellulose, lignin) and an overall, more available C supply for aquatic heterotrophs.

Nitrate is by far the most commonly reported N pollutant, and has increased dramatically, in Florida's major springs during the last 30 years (Scott *et al*, 2004). With this trend, many springs

are increasingly becoming P limited (Figure 12) (Inglett *et al.* 2007; Frazer *et al.* 2001). Aside from effects of incomplete denitrification (producing  $\text{NO}_2^-$  and potentially  $\text{N}_2\text{O}$ ), it is currently uncertain if high levels of  $\text{NO}_3^-$  can have any other adverse impacts to biogeochemical processes.

### Phosphorus Impacts

In general, many springs already have high amounts of bioavailable P with SRP levels  $>80$  ppb. High levels of bioavailable P lead to the general observation of low water column N:P ratios in most springs (i.e., those unaffected by  $\text{NO}_3^-$  pollution) (Figure 12). As in other aquatic systems, elevated P levels should result in higher overall productivity, or if limited by some other nutrient (or light) a higher potential productivity level. For this reason, P availability may determine the overall threshold response of a given spring system to another nutrient, such as N. This response may be visible only through increased productivity of certain spring species; however, in this manner, high-P springs may demonstrate a greater response (either through greater overall production or that of individual species) or be more susceptible to increased N.



**Figure 12** – Ratios of dissolved inorganic N:P and total N:P (mass basis) in the Wekiva river in relation to theoretical boundary between conditions of N limitation and conditions co-limited by N and P. (From Inglett *et al.* 2007)

Sediments in spring systems have the capability to store P as Ca-P or Fe-P complexes, and thus could be a reservoir of P that becomes mobilized upon increased levels of N. Fluctuations in water column P levels will determine if the sediments act as a sink or source, as low water

column P levels will favor flux of sedimentary P. The response of some systems to additions of P has been observed as a decrease in macrophytes chlorophyll levels. This may reflect changes in the microbial composition or the stoichiometric balance of N:P and C:P which could shift the dominance of autotrophs to more heterotrophic communities. Increased heterotrophy would have cascading type effects on the balance of aerobic/anaerobic conditions, and thus, would also effect the cycling of redox-sensitive elements such as N, P, Fe, and S.

### **Sulfur Impacts**

It is uncertain what role S may be playing in the biogeochemistry of Florida springs. Sulfur can be significant as an electron acceptor (as  $\text{SO}_4^{2-}$ ) with a toxic byproduct of  $\text{S}^{2-}$ . Also,  $\text{S}^{2-}$  can become a powerful electron donor for particular organism groups and can diversify the pathways for  $\text{NO}_3^-$  removal through denitrification. Furthermore, the impacts of sulfur may not be immediately visible from data collected at the boil/vent. The oxidation pathways and processes may develop slowly following groundwater emergence and may be temporally variable. These effects may also be spatially localized to zones of significant S input or transformation such as the mixing of spring water with contrasting water high in S (e.g., that observed when tributaries meet).

## **SUMMARY AND CONCLUSIONS**

Based on the above discussion, springs are clearly dynamic systems with a myriad of potential biogeochemical processes/reactions and interactions among elemental cycles. Although the majority of these basic biogeochemical processes have been identified in other aquatic systems, many may not be universally applied to spring systems because of their unique features including karst-derived water chemistry, and relatively constant temperature and flow conditions. A diversity of conditions within spring systems also gives rise to numerous microbially active zones where conditions may exist for as yet unexplored processes and pathways.

Nutrient cycles in spring systems are dominated by the conversion of inorganic to organic forms. Organic matter (dissolved and particulate forms) is therefore an important component of the spring system as it is both an internal supply of nutrients and a form of nutrient export. Carbon is also the basis of many other anaerobic biogeochemical reactions, and is thus an important determinant in the cycling of other nutrients including nitrogen, phosphorus, and metals. Despite this importance, organic matter distributions, concentrations, and quality types within spring systems are relatively unstudied, but may have an importance for how individual springs process and store nutrients. Also, aquatic plant management practices can be a contribution of organic matter deposition in spring runs (affecting internal nutrient cycling); however, the importance of the process is undetermined.

Nitrogen has been a nutrient of great interest in the Florida springs due to its trend of increasing concentrations in the springs which are associated to changes in spring appearance, especially increases in algae. In addition to external loads of N through  $\text{NO}_3^-$ , nitrogen fixation is an important processes contributing to N, but is also currently unstudied in Florida springs. There are several pathways which can act to convert  $\text{NO}_3^-$  (reducing its concentration), with the dominant most likely being heterotrophic denitrification or plant assimilation. The importance

of these two pathways will largely determine if a spring is a sink or source of N; however, current studies have not attempted to partition N flow through each pathway. High flow rates and patchy distributions of organic matter also indicate that conditions in many springs could support several alternative (novel) pathways of  $\text{NO}_3^-$  reduction, but again, these have received no attention.

Phosphorus may also play a key role in regulating species composition or productivity in a spring system. A variety of biotic and abiotic processes act to determine the biogeochemical cycling of phosphorus in spring systems. The generally high calcium content of many spring waters leads to the potential for accumulation of Ca-P compounds associated with biotic growth and mineral forms in sediments. Organic matter and associated enzymatic hydrolysis of organic P compounds is also another key mechanism regulating both accumulation and bioavailability of phosphorus in springs. Despite the significance of these processes; however, the general lack of studies focusing on sediment properties currently limits our understanding of P cycling, or its importance as an internal nutrient supply mechanism.

Several other elements could have importance for nutrient cycling in spring systems including sulfur and iron. Iron participates in redox reactions that determine both its form and solubility/mobility and its particular interaction with  $\text{PO}_4^{3-}$  (affecting P availability). Iron is also significant as a potential limit to algal growth in certain highly alkaline systems. Like iron, sulfur can undergo dynamic transformations through redox processes in alternating aerobic/anaerobic systems like springs. Presence of sulfide is likely only a problem (toxicity) in certain sulfur spring systems; however, there is a possibility that sulfide-driven denitrification may be a significant process reducing  $\text{NO}_3^-$  levels in some aquifers and in some spring runs.

### **Management and Policy Implications**

Nutrient enrichment of Florida springs is major concern. The steady increase in nutrient concentrations observed in many Florida springs during the past four decades threatens the economic and ecological value of these springs. Nonpoint sources of nutrients dominate eutrophication processes of many Florida springs. Thus, most recommendations involve the use of alternative land management practices in the watershed in an effort to reduce the overall load to these springs. The key management questions often asked are: (1) Will springs respond to external nutrient load reduction?, and (2) If so, how long will it take for these systems to recover and reach their background condition?

The question of system impact is important for management of spring systems. Nutrient limitation and biogeochemical processes are a very interactive to determine the biotic response of a given spring to external nutrient loads. The chemistry of spring source water is crucial to determine the types of metabolic pathways and diversity of organisms that will populate/dominate a spring ecosystem. In particular, microbial communities will likely demonstrate responses to changes in nutrient conditions immediately through changes in species or functional groups and through activities of enzymes which are produced in response to their external chemical environment. In this way, monitoring of biogeochemical processes may serve as an early warning indicator of spring health.

Much of the nutrients added to springs can be retained within the system through the accumulation of large reserves in detrital tissue and/or reactive forms in sediments. These nutrient stores can serve as a nutrient source for long period of time, even after external loads are reduced. As discussed in this paper, various biogeochemical processes in sediment and water column regulate the mobility and reactivity of stored nutrients. The cycling of nutrients can extend the time required for springs to reach their original condition, with a lag time from a few years to several decades, depending on the extent of internal nutrient supply. This lag time for recovery should be considered in developing management strategies and monitoring efforts, where environmental managers must recognize that the recovery process is not immediate, even despite successful reductions in external loads.

To determine the role of internal nutrient supply from sediments, it is important to document storages and fluxes of nutrients between sediment, vegetation, and water column. At this time little or no information is available on internal cycling of nutrients and its overall role in regulating eutrophication process. When considering internal cycling of nutrients in springs, several issues need be addressed. These issues are: (1) what is the relative bioavailability of various nutrient forms stored in sediments and water column? (2) how stable are the stored nutrients and under what conditions could they be released back into the water column? and (3) how long could the stored nutrients serve as a source to the water column to maintain eutrophic conditions, once external loads are reduced? Thus, monitoring internal nutrient storages on a regular basis is equally as important as monitoring external nutrient loads. Such information will be useful to the state's Department of Environmental Protection as it attempts to establish nutrient criteria and Total Maximum Daily Loads (TMDLs) for springs.

## **RESEARCH NEEDS**

Biogeochemistry is one of the least studied aspects of spring systems. The complexity of biogeochemical pathways, the complexity of the high energy/lotic spring environment, and the diversity of spring water types makes it difficult to form any wide-ranging generalizations. For this reason, biogeochemistry is also one of the aspects of spring systems requiring additional effort to increase our understanding of spring nutrient impacts. Based on the review of literature presented, the following are our recommendations for research related to the effects and consequences of nutrients in calcareous spring systems.

### **Specific Research Recommendations**

#### **Biogeochemical Characterization**

There is general lack of data on characterization of spring systems for biogeochemical purposes. Characterization data and long term measurements of water chemistry are available; however, the addition of some parameters to this monitoring are warranted, including sulfide ( $S^2$ ) and reduced iron (Fe(II)). Also, there is a large uncertainty in the function of sediments for storage and cycling of nutrients. Identification of biogeochemically active zones will depend on knowledge of sediment mineralogy, organic matter contents and qualities, and stratigraphic morphology. For this reason, data should also be collected to characterize the biogeochemical properties of spring run sediments including basic nutrient chemistry, mineralogy, and potential

storages of nutrients in the sediments of most springs/runs. Better mapping of spring run sediment characteristics can also help improve modeling of nutrient dynamics within a given spring run (including sediment function as sink or source of nutrients).

### **Source and Fate of N**

Research is needed to understand the fate of N within the spring system. This would include understanding of biotic uptake processes (algal/macrophyte uptake), importance of different N sources ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , DON), and the occurrence, rates and importance of biological  $\text{N}_2$ -fixation. Whole spring run N budgets should be performed to better isolate the processes responsible for N decline, and these data put in the context of nutrient limitation of systems of differing geology, channel morphology, water chemistry, and biotic productivity. Given the potential for structuring aquatic microbial communities, the presence and effects of potential DON sources (including urea) should be investigated in relation to algal composition, microbial activity, and community nutrient limitation. Potential pathways of  $\text{NO}_3^-$  loss should also be investigated, including identification of hotspots of denitrification as well as the potential occurrence and conditions regulating any novel N loss pathways (e.g., lithotrophic Fe(II)/ $\text{S}^{2-}$  oxidation coupled to denitrification, or anammox).

A variety of techniques can be employed to help answer some of these questions regarding N cycling and fate in spring systems. Determination of system N uptake can be done in small batch or whole stream segments (Saunders 2007; Wetland Solutions, Inc. 2005). Relative estimates of enzyme activities are simple to conduct and provide information regarding spatial or temporal distribution of processes (Inglett *et al.* 2007). Other enzyme assays can be used to provide quantitative estimates of N processes such as the determination of denitrification using the traditional acetylene inhibition assay (DEA technique; Inglett *et al.* 2007) or  $\text{N}_2$  fixation assays (Inglett *et al.* 2004). It is well known that natural abundance level stable N isotopic methods can be used to assess sources of N in spring systems; however, the analysis of  $\delta^{15}\text{N}$  can also provide information on rates of N uptake or denitrification (De Brabandere *et al.* 2007). Experiments utilizing tracer levels of  $^{15}\text{N}$  can also provide useful identification of N pathways (e.g., novel  $\text{NO}_3^-$  reduction pathways) as well as quantitative information of pathways and flows (e.g.,  $\text{N}_2$  fixation, N uptake, and denitrification).

### **Carbon Dynamics**

Carbon sources and distribution play a key role in structuring the biogeochemistry of the spring system, therefore better understanding is needed regarding the sources and processing of C in spring systems. This would include identification and quantification of autochthonous and allochthonous C sources (quantities and qualities), availability and cycling of different C types, and investigation of the importance of different C sources for respiration processes and denitrification. The role of aquatic plant management in the distribution and fate of C within particular systems is also of significant concern as it constitutes a significant source of C and nutrients within the system.

## **Sulfur Distribution and Function**

Little attention is given to sulfur contents of ground waters, except where smell or taste issues are concerned; however, S may be a key element regulating several biogeochemical cycles including N and P. For this reason, more emphasis should be given to S as it relates to biogeochemical processes including its distribution and redox state in spring environments (i.e., locations of oxidized and reduced S accumulations). One of the more important aspects of the uncertain role of sulfur is its potential involvement in regulating N and P biogeochemistry, therefore, studies linking redox processes with denitrification or P flux from sediments are potentially useful for elucidating the functioning of S in some nutrient-impacted spring systems.

## **Coupled Biogeochemical Cycles**

Identifying and understanding biogeochemical pathways is of critical importance; however, as should be evident from this review, biogeochemical processes do not act independently in natural systems. From the basic concept of organic matter and biotic productivity, it can be seen that elements (and thus elemental cycles) are tied together (or coupled) so that the pathways of one cycle inherently affect the pathways of another element.

Currently, there have been no attempts to understand the coupled nature of cycles (N and P; S, N, and P; S and trace metals) in spring systems. Not only could this have important implications for understanding both the localized effects of nutrients on the biota (e.g., questions of N versus P limitation), but it could also have application to modeling the function of these systems as sinks, sources or transformers of nutrients and C at the landscape scale. For this reason, there should be increased focus on assessing the potential for linkages between biogeochemical cycles (C, N, P, and S) as they relate to landscape patterns of geomorphology, species composition/productivity, land use, and nutrient impact. In addition, data sets (both current and future) should incorporate the concepts of coupled biogeochemical cycles when collecting data to assess spatial and temporal scales.

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