Review of nitrogen and phosphorus metabolism in seagrasses

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Abstract

Within the past few decades, major losses of seagrass habitats in coastal waters impacted by cultural eutrophication have been documented worldwide. In confronting a pressing need to improve the management and protection of seagrass meadows, surprisingly little is known about the basic nutritional physiology of these critical habitat species, or the physiological mechanisms that control their responses to N and P gradients. The limited available evidence to date already has revealed, for some seagrass species such as the north temperate dominant *Zostera marina*, unusual responses to nutrient enrichment in comparison to other vascular plants. Seagrasses derive N and P from sediment pore water (especially ammonium) and the water column (most nitrate). The importance of leaves versus roots in nutrient acquisition depends, in part, on the enrichment conditions. For example, a shift from reliance on sediment pore water to increased reliance on the overlying water for N and P supplies has been observed under progressive water-column nutrient enrichment. Seagrasses may be N-limited in nutrient-poor waters with sandy or (less so) organic sediments, and P-limited in carbonate sediments. On the basis of data from few species, seagrasses appear to have active uptake systems for NO_3^- and PO_4^{3-} , but NH_4^+ uptake may involve both low- and high-affinity systems. P_i uptake affinities reported thus far are much lower than values for active ammonium uptake, but comparable to values for nitrate uptake by leaf tissues. Beyond such basic information, seagrass species have shown considerable variation in nutritional response. Dominance of acropetal versus basipetal nutrient translocation appears to vary among species as an innate trait. While some species follow classic Michaelis–Menten kinetics for N_i uptake, others have exhibited sustained linear uptake with limited or negligible product feedback inhibition, perhaps in adaptation to oligotrophic environments. *Zostera marina* also is able to maintain nitrate reductase (NR) activity during dark periods if adequate carbohydrate reserves and substrate are available. Thus, this species can respond to nitrate pulses throughout a diel cycle, rather than being limited as most plants to nitrate uptake during the light period. Further adaptations may have occurred for seagrasses in extremely nitrate-depauperate conditions. For example, *Halophila decipiens* and *H. stipulacea* lack inducible NR and apparently have lost the ability to reduce nitrate; and a biphasic rather than hyperbolic P_i uptake curve, with ‘surge’ uptake, has been described for *Zostera noltii*. Many seagrasses respond favorably to low or moderate N and/or P enrichment. However, excessive N_i loading to the water column can inhibit seagrass growth and survival, not only as an indirect effect by stimulating algal overgrowth and associated light

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reduction, but—for some species—as a direct physiological effect. The latter direct impact has been most pronounced for plants growing in sandy (nutrient-poor) sediments, and is exacerbated by elevated temperatures and/or light reduction. Ammonia toxicity, known for many vascular plants, has been reported in seagrasses *Ruppia drepanensis* and *Z. marina* (125 μM water-column NH_4^+ , 5 weeks). *Z. marina* has shown to be inhibited, as well, by pulsed water-column nitrate enrichment (as low as 3.5–7 μM NO_3^- , 3–5 weeks), which is actively taken up without apparent product feedback inhibition. Inhibition by elevated nitrate has also been reported, with description of the underlying physiological mechanisms, in certain macroalgae and microalgae. In *Z. marina*, this effect has been related to the high, sustained energy demands of nitrate uptake, and to inducement of internal carbon limitation by the concomitant ‘carbon drain’ into amino acid assimilation. In contrast, nitrate enrichment can stimulate growth of *Z. marina* when the sediment, rather than the water column, is the source. Because seagrass species have shown considerable variation in nutritional response, inferences about one well-studied species, from one geographic location, should not be applied a priori to that species in other regions or to seagrasses in general. Most of the available information has been obtained from study of a few species, and the basic nutritional physiology of many seagrasses remains to be examined and compared across geographic regions. Nonetheless, the relatively recent gains in general understanding about the physiological responses of some seagrass species to nutrient gradients already have proven valuable in both basic and applied research. For example, physiological variables such as tissue C:N:P content have begun to be developed as integrative indicators of nutrient conditions and anthropogenic nutrient enrichment. To strengthen insights for management strategies to optimize seagrass survival in coastal waters adjacent to exponential human population growth and associated nutrient inputs, additional emphasis is critically needed to assess the role of variable *interactions*—among inorganic as well as organic N, P and C, environmental factors such as temperature, light, and other community components—in controlling the physiology, growth and survival of these ecologically important marine angiosperms. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Seagrasses are highly productive marine angiosperms that grow in shallow coastal marine waters, often in sheltered embayments and lagoons which are poorly flushed and sensitive to nutrient loading from adjacent human population growth (Harlin, 1993). Seagrasses provide critical habitat and a nutritional base for finfish, shellfish, waterfowl, and herbivorous mammals (Phillips and Meñez, 1988; Klumpp et al., 1989). In the past few decades, major declines in seagrass meadows have been reported worldwide, attributed to light reduction from land disturbance and sediment loading/resuspension, and light reduction from algal overgrowth that is stimulated by cultural eutrophication (Harlin, 1993; Morris and Tomasko, 1993). Under accelerated eutrophication, the severity of sediment hypoxia/anoxia and sulfide reduction that are encountered by seagrass root-rhizome tissues increase, as well (Mackin and Swider, 1989; Dauer et al., 1993; Goodman et al., 1995).

Light generally has been considered to be the primary resource that limits the growth

of most seagrass species (McRoy, 1974; Phillips and Meñez, 1988). However, this concept has become dogma, and should not be applied a priori to seagrass systems (Burkholder et al., 1992). Sediment pore waters can provide high supplies of most nutrients for seagrasses (except carbon), with the water column as an additional source (McRoy et al., 1972; Short and McRoy, 1984; Harlin, 1993). Thus, nutrients are commonly regarded as secondary factors limiting growth. The relative importance of nutrients as limiting factors increases for plants growing in sandy, nutrient-poor substrata and nutrient-poor waters. As a generalization, seagrasses are regarded as N-limited when growing in sandy or organic sediments, and as P-limited in carbonate sediments (Short, 1987; Short et al., 1990; Williams, 1990; Kenworthy and Fonseca, 1992; but see Zimmerman et al., 1987). In certain conditions, seagrasses may be co-limited by N and P (Thursby, 1984; Udy and Dennison, 1997a).

Although the influence of light availability on seagrass growth has been examined at length (e.g., Dennison et al., 1993), much less emphasis has been directed toward understanding the nutritional ecology of seagrasses, or their physiological responses to nitrogen and phosphorus enrichments (Burkholder et al., 1992). A scan of the literature in 1990, for example, yielded few published studies on aspects of nitrogen uptake and metabolism by *Zostera marina*, the dominant seagrass species in temperate and arctic regions of North America. Within the past decade, however, nutritional ecology and physiology have become more of a central focus in seagrass research. Here we synthesize the available information known about nitrogen and phosphorus metabolism of seagrasses, and the interplay between N, P, carbon, and environmental factors such as temperature and light in controlling the growth and survival of these ecologically important marine angiosperms.

2. N and P regimes in seagrass ecosystems

Reports of N limitation in seagrasses often seem counter-intuitive, since sediment inorganic nitrogen availability (N_i , as NO_3^- and as ammonia which is mostly ionized as NH_4^+) would appear to be sufficient to sustain active, unlimited growth (Zimmerman et al., 1987). Nonetheless, many studies with N fertilization have demonstrated increased seagrass growth with increased N_i (Table 1). One possible explanation for N-limited growth in natural systems is competition between seagrasses and other community flora such as micro- and macroalgae (Harlin, 1993), as well as microheterotrophs which can assimilate high proportions of regenerated ammonium (40% or more; Iizumi et al., 1982; Thayer et al., 1984). Ammonium also adsorbs to sediment and detrital particles (Simon, 1989). Thus, although ammonium levels may appear to be relatively high, the actual ammonium available for seagrasses may be substantially lower (Thayer et al., 1984).

Nitrate is the primary form of N_i available to terrestrial plants (Wu et al., 1998; Jenkins et al., 1999). However, in the water-saturated, hypoxic or anoxic sediments that characterize seagrass habitats, ammonium is considered to be the dominant form of N_i (Riley and Chester, 1989; Stapel et al., 1996; Stumm and Morgan, 1996). Levels range from 1 to 180 μM NH_4^+ in sediment pore water, depending on sediment characteristics (especially percent organic matter) and community composition (Bulthuis and Woekerl-

Table 1

Overview of seagrass responses to nutrient enrichment and/or eutrophication events^a

Species	Response	Mechanism	Nutrient	Source
Temperate				
<i>Heterozostera tasmanica</i>	Increased growth	N limitation	N & P	Bulthuis and Woekerling (1981)
<i>Heterozostera tasmanica</i>	Increased growth	N limitation	N	Bulthuis et al. (1992)
<i>Posidonia oceanica</i>	Increased growth	N & P limitation	N & P	Alcoverro et al. (1997)
<i>Ruppia drepanensis</i>	Die-off	Physiological	N	Santamaría et al. (1994)
<i>Ruppia maritima</i>	Increased growth	N limitation	N	Burkholder et al. (1994)
<i>Zostera capricorni</i>	Die-off	Light attenuation	N	Abal and Dennison (1996)
<i>Zostera capricorni</i>	Increased growth	N & P co-limitation	N & P	Udy and Dennison (1997a,b)
<i>Zostera marina</i>	Increased growth	N limitation	N	Short (1987)
<i>Zostera marina</i>	Increased growth	N limitation	N & P	Kenworthy and Fonseca (1992)
<i>Zostera marina</i>	Increased growth	N limitation	N	Pedersen and Borum (1993)
<i>Zostera marina</i>	Die-off	Physiological	N	Burkholder et al. (1992, 1994)
<i>Zostera marina</i>	Die-off	Epiphytes	N & P	Neckles et al. (1993)
<i>Zostera marina</i>	Die-off	Macroalgal growth	N	Boynton et al. (1996)
<i>Zostera marina</i>	Die-off	Light attenuation	N	Lent et al. (1995)
<i>Zostera marina</i>	Die-off	Light attenuation	N & P	de Casabianca et al. (1997)
<i>Zostera noltii</i>	Die-off	Light attenuation	N & P	de Casabianca et al. (1997)
Tropical/subtropical				
<i>Cymodocea nodosa</i>	Increased growth	P limitation	P	Pérez et al. (1994)
<i>Cymodocea serrulata</i>	No effect		N & P	Udy and Dennison (1997a,b)
<i>Enhalus acoroides</i>	Increased growth	N limitation	N & P	Agawin et al. (1996)
<i>Halodule uninervis</i>	Increased growth	N limitation	N & P	Udy and Dennison (1997a,b)
<i>Halodule wrightii</i>	Increased growth	N & P limitation	N & P	Powell et al. (1989)
<i>Cymodocea nodosa</i>	Increased growth	P limitation	P	Pérez et al. (1994)
<i>Cymodocea serrulata</i>	No effect		N & P	Udy and Dennison (1997a,b)
<i>Enhalus acoroides</i>	Increased growth	N limitation	N & P	Agawin et al. (1996)
<i>Halodule uninervis</i>	Increased growth	N limitation	N & P	Udy and Dennison (1997a,b)
<i>Halodule wrightii</i>	No effect		N & P	Kenworthy and Fonseca (1992)
<i>Halodule wrightii</i>	Increased growth	N limitation	N	Burkholder et al. (1994)
<i>Syringodium filiforme</i>	Increased growth	N limitation	N	Short et al. (1993)
<i>Syringodium filiforme</i>	Increased growth	P limitation	P	Short et al. (1990)
<i>Thalassia hemprichii</i>	Increased growth	P limitation	N & P	Agawin et al. (1996)
<i>Thalassia testudinum</i>	Die-off	Microbial associations	N	Durako and Moffler (1987)
<i>Thalassia testudinum</i>	Increased growth	N and P limitation	N & P	Powell et al. (1989)
<i>Thalassia testudinum</i>	Die-off	Macroalgal growth	N & P	McGlathery (1995)
<i>Thalassia testudinum</i>	Die-off	Light attenuation	N	Tomasko et al. (1996)

^a Data include seagrass species (temperate and tropical/subtropical), growth response, possible mechanisms for the observed responses, nutrients involved, and source.

ing, 1981; Fourqurean et al., 1992a; Lee and Dunton, 1999; Table 2). Total water-column N_i is typically less than 3 μM during light periods in habitats lacking appreciable land-based anthropogenic influence as freshwater inflow (Table 2; Tomasko and Lapointe, 1991; Dunton, 1996; but see Lee and Dunton, 1999). Higher NH_4^+ in both the water column and sediment pore water is available (as regenerated N_i) at night on a diel basis, especially in warm seasons, because of increased decomposition from ecosystem metabolism and temporary low-oxygen conditions at the sediment–water

Table 2

Summary of water column and sediment nutrient concentrations observed in seagrass habitats, including species, ammonium, nitrate + nitrite, and phosphate (all nutrients in μM), location, and source^a

Species	NH_4^+	$\text{NO}_3^- + \text{NO}_2^-$	PO_4^{3-}	Location	Source
Water column					
Temperate					
<i>Phyllospadix torreyi</i>	0.42	0.40	–	S. California, USA	Terrados and Williams (1997)
<i>Posidonia australis</i>	0.47–0.5	0.08–0.1	0.13–0.25	Cockburn Sound, Australia	Silberstein et al. (1986)
<i>Zostera capricorni</i>	0.7	< 0.1	0.53	Moreton Bay, Australia	Boon (1986)
<i>Zostera marina</i>		up to 8	1.0	Chesapeake Bay, USA	Moore et al. (1996)
Tropical/subtropical					
<i>Cymodocea serrulata</i>	0.7	< 0.1	0.53	Moreton Bay, Australia	Boon (1986)
<i>Enhalus acoroides</i>	1.4	0.6	1.7	Indonesia	Erftemeijer (1994)
<i>Enhalus acoroides</i>	1.4	0.9	0.8	Indonesia	Erftemeijer (1994)
<i>Thalassia hemprichii</i>	1.4	0.9	0.8	Indonesia	Erftemeijer (1994)
<i>Thalassia hemprichii</i>	1–2.6	–	0.1–0.6	Indonesia	Stapel et al. (1996)
<i>Thalassia testudinum</i>	0–3.2	0.05–2.3	0–0.43	Florida Keys, USA	Tomasko and Lapointe (1991)
Sediment					
Temperate					
<i>Phyllospadix torreyi</i>	9.6	4.8	–	S. California, USA	Terrados and Williams (1997)
<i>Zostera capricorni</i>	6.7–11.5	–	0.7–6.3	Moreton Bay, Australia	Udy and Dennison (1997a,b)
<i>Zostera capricorni</i>	29–59	3–10	3–17	Moreton Bay, Australia	Boon (1986)
Tropical/subtropical					
<i>Cymodocea serrulata</i>	37–57	5–6	6–20	Moreton Bay, Australia	Boon (1986)
<i>Enhalus acoroides</i>	0.8	2.2	0.9	Indonesia	Erftemeijer and Middelburg (1995)
<i>Enhalus acoroides</i>	47–90	–	3.5–7	Indonesia	Erftemeijer and Middelburg (1993)
<i>Halodule wrightii</i>	10–50	–	–	Caribbean	Williams (1990)
<i>Halodule wrightii</i>	150–175	–	2–4	Florida Bay, USA	Fourqurean et al. (1992a,b)
<i>Syringodium filiforme</i>	10–50	–	–	Caribbean	Williams (1990)
<i>Thalassia hemprichii</i>	60–136	–	0.92–5.5	Indonesia	Stapel et al. (1996)
<i>Thalassia hemprichii</i>	0.8	2.2	0.9	Indonesia	Erftemeijer and Middelburg (1995)
<i>Thalassia hemprichii</i>	25–60	–	6–16	Indonesia	Erftemeijer and Middelburg (1993)
<i>Thalassia testudinum</i>	8–180	–	–	S. Texas, USA	Lee and Dunton (1999)
<i>Thalassia testudinum</i>	60–120	–	0.3–0.8	Florida Bay, USA	Fourqurean et al. (1992a,b)
<i>Thalassia testudinum</i>	10–50	–	–	Caribbean	Williams (1990)

^a Data are given as means, or as ranges if means were not available.

interface (Henriksen and Kemp, 1988). A considerable portion of the available N_i (NH_4^+) in seagrass beds can also be provided by cyanobacterial (blue–green algal) and eubacterial nitrogen fixation (Capone et al., 1979).

Nitrate supplies for most seagrasses are provided by leaf absorption from the water column (Terrados and Williams, 1997; Lee and Dunton, 1999), except for groundwater inflows that can contribute as much as $800 \mu\text{M NO}_3^- \text{N}$ to seagrass beds if influenced by septic effluent leachate or other anthropogenic sources (Maier and Pregnall, 1990; Harman et al., 1996). Leakage of oxygen from the lacunar system can create a microaerobic environment immediately adjacent to roots (Penhale and Wetzel, 1983), which can oxidize ammonia to nitrate as a mechanism for belowground tissue uptake of this highly soluble N_i form (Iizumi et al., 1980; Stumm and Morgan, 1996). However,

most nitrate is regarded as ‘new’ nitrogen for the system, contributed from anthropogenic rather than regenerated inputs (Glibert, 1988; Henriksen and Kemp, 1988). Aside from N_i sources, seagrasses can also utilize certain organic N substrates such as amino acids (product of decomposition) and urea (waste product from various fauna in seagrass communities; also contributed by poorly treated human and animal wastes; Vitousek et al., 1997; see below).

Phosphorus levels within seagrass habitats range from ca. 0.1 to 1.7 μM (as available dissolved phosphate, PO_4^{-3}P) in the water column, with higher concentrations in the sediment pore water (0.3–20 μM PO_4^{-3}P ; Table 2). Most P_i absorption for seagrasses is believed to occur through the roots (McRoy et al., 1972; but see below). Phosphate is highly biologically active, with a characteristically short residence time in the water column (Day et al., 1989). Like N_i , it is consumed by seagrasses, other community flora such as micro- and macroalgae (Harlin, 1993), and microheterotrophs (Gächter and Meyer, 1993; Slomp et al., 1993). And, like ammonium, higher levels of PO_4^{-3} are available in both the water column and sediment pore water at night on a diel basis, especially in warm seasons, because of increased decomposition and sediment release under low-oxygen conditions (Day et al., 1989). P_i is also highly insoluble and readily adsorbs to organic and inorganic particles (Froelich, 1988; Stumm and Morgan, 1996). In marine environments, especially in tropical and subtropical areas that are dominated by carbonate sediments, P_i can be effectively removed from access by seagrasses through formation of calcium phosphate complexes (e.g., $\text{Ca}_3(\text{PO}_4)_2$; Garrels et al., 1975; Stumm and Morgan, 1996).

3. Nitrogen physiology

The physiology for N_i assimilation to amino acids by plant tissues is complex, especially when the N_i form is nitrate, and involves various biochemical pathways that occur within the cytosol, the chloroplast, and the mitochondria (Fig. 1). Whereas nitrate is taken up via an active transport system (see below), ammonium uptake may be more complex. The electrochemical gradient between the apoplast and the symplast of plants traditionally has been viewed as a region of passive movement/uptake of small cations, including NH_4^+ , through membrane channels (Ourry et al., 1997). Recently, however, studies on agriculturally important plants have revealed active enzyme-like uptake responses to NH_4^+ , including substrate saturation at low NH_4^+ levels (e.g., Wang et al., 1993; Mäck and Tischner, 1994). Moreover, plants grown in low-N or N-free media have been shown initially to have low NH_4^+ uptake rates after NH_4^+ additions, followed by an induction-like response with increased uptake rates after several hours (Ourry et al., 1997). These data indicate that NH_4^+ uptake may involve two separate transport systems—a low-affinity system based on passive influx and efflux of NH_4^+ through membrane channels, and a high-affinity system requiring protein synthesis for formation of uniports (transmembrane transport protein that conveys a single species across the plasma membrane; Ourry et al., 1997). It is also possible, given the product feedback characteristic of many high-affinity systems (Goodwin and Mercer, 1983), that the

Fig. 1. Theoretical nutrient uptake curve, with parameters based on the Michaelis–Menten model for uptake kinetics (adapted from Cornish-Bowden, 1979). Parameters include $V_{\max}$ (maximum uptake rate), K_m (half-saturation constant = $1/2V_{\max}$), α (uptake affinity = $V_{\max}/K_m$), and $S_{\min}$ (minimum substrate level for uptake to occur).

assimilated NH_4^+ may exert a negative feedback on further uptake when tissue ammonia levels are high (Lee and Ayling, 1993).

Although most available information about N acquisition by seagrasses has emphasized N_i forms, a few seagrass species have been shown to be capable of organic N uptake as amino acids and urea (McRoy and Goering, 1974; Bird et al., 1998). Surprisingly, up to 20% of the total N supply in vascular plants has been reported to be derived from amino acids (Schobert and Komor, 1987; Bush, 1993). Amino acid uptake apparently requires H^+ ATPase pumps that promote membrane depolarization as well as alkalinization of the external medium (Ourry et al., 1997). Information on urea assimilation by seagrasses is extremely limited: McRoy and Goering (1974) showed urea uptake by field-collected *Zostera marina*, whereas Bird et al. (1998) reported that *Halophila decipiens* grown in tissue culture did not utilize urea.

3.1. N acquisition by above- and belowground tissues

Young, actively growing roots have been reported to take up most of the N absorbed by belowground tissues in seagrasses, with rhizomes functioning minimally in N_i uptake (Short and McRoy, 1984; Barnabas, 1991; Stapel et al., 1996). Although sediment pore water is generally considered to be the primary N_i source for seagrasses, recent evidence suggests that uptake of both N and P by belowground tissues can be limited by diffusion, and that roots may lack the capacity to support the total nutrient requirement (Stapel et al., 1996; Lee and Dunton, 1999). Thus, experiments by Lee and Dunton (1999)

indicated that about 50% of the nitrogen uptake in *Thalassia testudinum* occurs through the leaves. Other studies suggest that from 30 to 90% of the N requirement in *Zostera marina* can be supplied by the water column (Iizumi and Hattori, 1982; Short and McRoy, 1984; Pedersen and Borum, 1992, 1993). In extreme cases—for example, on rock substrata (as for *Phyllospadix torreyi* and, to some extent, *Amphibolis antarctica*)—nearly all nutrient absorption occurs in the leaves (Terrados and Williams, 1997).

The role of leaves in nutrient acquisition may partly be explained by the relatively high nutrient uptake affinities of these tissues. Many seagrasses can assimilate N_i through their leaves at extremely low levels (Pedersen et al., 1997; Lee and Dunton, 1999). This response may reflect an adaptation of seagrasses to oligotrophic environments (Burkholder et al., 1994). It allows plants to acquire pulsed low-level N_i as it enters the system, while also absorbing nutrients that diffuse from the sediments, or that are released from detrital decomposition (Paling and McComb, 1994; Stapel et al., 1996).

In *Zostera marina*, N (as N_i and urea) assimilated by roots can be moved to aboveground tissues (acropetal or root-to-shoot translocation; McRoy and Goering, 1974). Conversely, assimilated N_i from leaves can be translocated to belowground tissues (basipetal or shoot-to-root translocation; Iizumi and Hattori, 1982). Ammonium uptake by aboveground tissues is reportedly unaffected by NH_4^+ concentrations available to the roots, even at sediment pore water concentrations in excess of 150 μM (Thursby and Harlin, 1982). In contrast, maximum rates of root ammonium uptake were substantially compromised (decreased from 211 to 48 $\mu mol\ g^{-1}\ dry\ weight\ h^{-1}$) during periods when leaves were exposed to increased ammonia (15–30 μM ; Thursby and Harlin, 1982). It was hypothesized that the observed response was associated with higher basipetal translocation of N products relative to acropetal translocation.

These characteristics of N uptake by *Z. marina* tissues may explain, in part, the sustained uptake of water-column nitrate enrichment by *Z. marina* that has been observed in other studies (e.g., Roth and Pregnull, 1988; Burkholder et al., 1994—indicated by nitrate reductase activity; see below), even when sediment N_i (as ammonia) appears to be at levels required for optimal growth (Thursby and Harlin, 1982; but see Roth and Pregnull, 1988, below). In contrast to the somewhat independent response of *Z. marina* leaves to belowground tissue N_i availability, uptake of ammonium by *Ruppia maritima* leaves has been described to be strongly affected by ammonium supplies to the roots (Thursby and Harlin, 1984). Acropetal translocation may be dominant in this plant, or roots may be unaffected by internal whole-plant ammonium status (Thursby and Harlin, 1984).

3.2. Nitrogen uptake kinetics

For seagrass species that follow Michaelis–Menten kinetics in N_i absorption, the uptake affinity coefficient (α , defined as the initial slope of the V versus S curve; Table 3, Fig. 2) is useful in evaluating the efficiency of nutrient uptake within the concentration range characteristic of natural conditions (e.g., Stapel et al., 1996). In leaf tissues, uptake affinities for N_i are highly variable, ranging from 0.5 to 5.5 for NH_4^+ and from 0.15 to 1.7 for NO_3^- , indicating higher affinity for NH_4^+ than for NO_3^- (Table 3). Moreover, in

Table 3

Nutrient uptake parameters reported in seagrass species based on the Michaelis–Menten model for uptake kinetics, including tissue (leaf, root), nutrient, $V_{\max}$ (maximum uptake rate, $\mu\text{mol g}^{-1}$ dry weight h^{-1}), K_m (half-saturation constant, μM), and α (uptake affinity = $V_{\max}/K_m$)

Species	Nutrient	$V_{\max}$	K_m	α	Source
Leaf					
Temperate					
<i>Amphibolis antarctica</i>	NH_4^+	5.9–43.1	9.5–74.3	0.6–0.8	Pedersen et al. (1997)
<i>Phyllospadix torreyi</i>	NH_4^+	95.6–204.3	9.3–33.9	–	Terrados and Williams (1997)
<i>Phyllospadix torreyi</i>	NO_3^-	24.9–75.4	4.4–17.0	–	Terrados and Williams (1997)
<i>Ruppia maritima</i>	NH_4^+	243–270	9.0–17.7	5.5	Thursby and Harlin (1984)
<i>Zostera marina</i>	NH_4^+	20.5	9.2	2.2	Thursby and Harlin (1982)
<i>Zostera marina</i>	NO_3^-	–	23	–	Iizumi and Hattori (1982)
<i>Zostera noltii</i> (excised) ^a	PO_4^{3-}	7.0	10	0.7	Pérez-Lloréns and Niell (1995)
<i>Zostera noltii</i> ^a	PO_4^{3-}	43	12.1	1.1	Pérez-Lloréns and Niell (1995)
Tropical/subtropical					
<i>Thalassia hemprichii</i> ^a	PO_4^{3-}	2.2–3.2	7.7–15	0.12–0.19	Stapel et al. (1996)
<i>Thalassia hemprichii</i>	NH_4^+	32–37	21–60	0.52–0.85	Stapel et al. (1996)
<i>Thalassia testudinum</i>	NH_4^+	8.3–16.4	7.6–15	0.57–2.82	Lee and Dunton (1999)
<i>Thalassia testudinum</i>	NO_3^-	3.7–6.5	2.2–38.5	0.15–1.68	Lee and Dunton (1999)
Root					
Temperate					
<i>Amphibolis antarctica</i>	NH_4^+	1.1	4.7	0.2	Pedersen et al. (1997)
<i>Ruppia maritima</i>	NH_4^+	48–56	2.8–12.6	20.1	Thursby and Harlin (1984)
<i>Zostera marina</i>	NH_4^+	211	104	0.5	Thursby and Harlin (1982)
<i>Zostera marina</i>	NH_4^+	–	30	–	Iizumi and Hattori (1982)
Tropical/subtropical					
<i>Thalassia testudinum</i>	NH_4^+	7.9–73.3	34.4–765.5	0.03–0.30	Lee and Dunton (1999)

^a Note: minimum substrate level ($S_{\min}$) was also available for *Zostera noltii* (excised leaf, 2.5 μM ; leaf, 2.6 μM) and *Thalassia hemprichii* (leaf, 0.7–1.1 μM).

many seagrass species (e.g., *Amphibolis antarctica*, *Thalassia testudinum*, *Zostera marina*), calculated uptake affinities for NH_4^+ are higher in leaves than in roots (Table 3; root values highly variable, ranging from 0.03 to >20). Thus, seagrass leaves tend to be more efficient than roots in absorbing low levels of ammonium—a response that likely reflects the low levels of NH_4^+ in the water column relative to the sediments (Lee and Dunton, 1999).

Seagrass leaves generally have higher uptake rates for NH_4^+ (between 5 and 270 $\mu\text{mol g}^{-1}$ dry weight h^{-1}) than for NO_3^- (3.7 to 75 $\mu\text{mol g}^{-1}$ dry weight h^{-1}). This trend has been shown for *Amphibolis antarctica*, *Phyllospadix torreyi*, *Thalassia testudinum* and *Zostera marina* (Short and McRoy, 1984; Paling and McComb, 1994; Terrados and Williams, 1997; Lee and Dunton, 1999). Although these rates vary seasonally, Lee and Dunton (1999) reported negligible variations in $V_{\max}$ and K_m in light versus dark assimilation of NH_4^+ (roots and leaves) or NO_3^- (leaves) by *T. testudinum*. Nitrate uptake by *Z. marina* in response to pulsed inputs has been experimentally shown in both darkness and light, as well (Touchette, 1999; see below). Thus, some seagrasses can develop the capability to absorb N_i regardless of light conditions. This characteristic

Fig. 2. Reactions involved in nitrate uptake and assimilation in plant cells. Uptake and assimilation of nitrate as well as ammonium occur primarily in the cytosol and plastids (chloroplasts, mitochondria). As nitrate enters the cell, it can be stored in a vacuole (not shown) or reduced in the cytosol by the enzyme nitrate reductase (NR). The product, nitrite, enters the plastids (usually the chloroplasts) where it is further reduced to ammonium by the enzyme nitrite reductase (NiR). Ammonium then enters the GS/GOGAT cycle, where glutamate is aminated by the enzyme glutamine synthetase (GS) to synthesize glutamine. The addition of carbon skeletons (α -ketoglutarate) allows transamination by glutamate synthase (GOGAT) to produce two glutamate molecules. One of these can be recycled through the GS/GOGAT pathway, whereas the other can be used to form more complex amino acids (adapted from Ferrario-Méry et al., 1997).

would be especially advantageous for seagrasses in oligotrophic habitats, by enabling them to utilize N_i supplies from nocturnal storm events or other source of nutrient pulses.

The capacity for nutrient uptake ($V_{\max}$, which can be considered as an indication of the number of active uptake sites; Thursby and Harlin, 1984) also varies in response to changing nutrient conditions. In *Ruppia maritima*, for example, $V_{\max}$ was significantly lower in N-starved than in N-replete plants (Thursby and Harlin, 1984). This observation may have indicated the presence of a feedback inhibition mechanism for plants growing in eutrophic conditions. In *Zostera marina* leaves, a similar adjustment in $V_{\max}$ was reported following long-term exposure (e.g., 24 h) to increased NH_4^+ (Short and McRoy, 1984). The latter research involved time-series incubations on plants in situ at a location that was described as N-limited; thus, the biphasic NH_4^+ uptake response may have been symptomatic of initial N starvation, perhaps followed by product feedback inhibition.

Although some seagrasses have followed Michaelis–Menten kinetics for N_i uptake, other species have shown more variable response; or, the same seagrasses have not followed Michaelis–Menten uptake kinetics under other conditions. For example, seedlings of the seagrass *Amphibolis antarctica* showed a direct linear relationship between uptake rates and N_i concentrations (from 0.3 to 36.0 μM of NO_3^- and NH_4^+) and, thus, a Michaelis–Menten analytical approach was not applicable (Paling and McComb, 1994). The seedlings survived and grew as leaf clusters with little or no root development, so that N_i uptake was restricted mostly to leaves until the plants had matured. Given this inability to directly utilize sediment N_i , it is reasonable that *A. antarctica* seedlings would maintain linear uptake of N_i at high water-column levels when such pulses became available.

Studies of N_i assimilation in several seagrass species have shown limited or negligible product feedback inhibition. For example, NH_4^+ uptake kinetics in the seagrass *Thalassia hemprichii* indicated no evidence of a feedback inhibition mechanism (Stapel et al., 1996). The authors of that study hypothesized that the lack of this mechanism was indicative of an adaptation to oligotrophic environments. This lack of an apparent substrate saturation or product feedback inhibition may provide insights about adaptive responses of seagrasses to changing nutrient regimes. Research by Roth and Pregannal (1988) illustrates this point: *Zostera marina* was collected from sandy (low N) versus organic (high N) sediments and low-N overlying waters. Nitrate uptake was compared under N-enriched conditions (laboratory trials, 6 days, 200 μM NO_3^-). The plants grown in sandy sediments showed a linear response in nitrate uptake, with no evidence of product feedback inhibition over time. A linear response in nitrate uptake was also shown by the plants from N-rich sediments, but uptake was significantly lower. The data indicate that the low N-adapted plants had conformed to maximize their response to water-column NO_3^- enrichment, relative to the plants that had been growing in sediment N-enriched conditions.

3.3. Ammonium assimilation

The glutamine synthetase (GS)/glutamate synthase (glutamine-oxoglutarate amidotransferase or GOGAT) pathway of ammonium incorporation (Goodwin and Mercer, 1983) is the primary biochemical cycle involved in ammonium assimilation. This pathway involves initial incorporation of ammonium to glutamate by GS, using ATP to catalyze formation of glutamine (localized in the cytosol and chloroplast of photosynthetic tissues, and in the cytosol of roots; Emes and Fowler, 1979; Oaks and Hirel, 1985). Glutamate may then be regenerated from glutamine and α -ketoglutarate by the enzyme GOGAT (localized in the chloroplast of photosynthetic tissues, and in root plastids; Oaks and Hirel, 1985), for use in production of new amino acids or in completing the GS/GOGAT pathway as the initial substrate for ammonium assimilation. The GS/GOGAT system is also involved in secondary assimilation of ammonium that can be released from glycine during photorespiration—a mechanism that is regarded as essential for N conservation within plants (Goodwin and Mercer, 1983).

Ammonium, partly because of its toxicity as a decouplet of photophosphorylation in chloroplasts (Marschner, 1995), does not accumulate in healthy seagrass tissues, thus

indicating rapid assimilation into organic compounds (Touchette, 1999). Most of the research on ammonium assimilation in higher plants has focused on GS activity because it is believed to be the key enzyme involved in synthesis of amino acids (Temple and Sengupta-Gopalan, 1997). GS activity of both belowground (e.g., *Zostera marina*; Kraemer and Alberte, 1993) and aboveground tissues (e.g., *Posidonia oceanica*; Kraemer et al., 1997) decreases with tissue age. Moreover, GS activity is higher in above- than in belowground tissues (e.g., 50-fold higher in leaves than in roots; Kraemer et al., 1997). These data, like the data for N uptake kinetics, indicate the importance of aboveground tissues in N assimilation.

GS activity in seagrasses is highly influenced by the surrounding environment. For example, Kraemer et al. (1997) demonstrated that leaf GS activity in *P. oceanica* varies with depth, season, and location. Highest GS activity was observed during active growth periods as expected, and for plants in deeper waters (for both *Z. marina* and *P. oceanica*; Pregnall et al., 1987; Kraemer et al., 1997). Moreover, as in terrestrial plants (e.g., Kozaki et al., 1991; Seith et al., 1994; Sukanya et al., 1994), increasing external N_i supplies can promote significant increases in GS gene expression and activity in seagrasses (for *Z. marina* and *P. oceanica*; Pregnall et al., 1987; Kraemer et al., 1997). Kraemer et al. (1997) suggested that in vivo GS activity in *P. oceanica* may be limited by the availability of the substrate (NH_4^+), carbon skeletons (e.g., glutamate or α -ketoglutarate), and/or energy.

Temperature, hypoxia, and light availability (i.e., intensity and H_{sat} , the duration of the daily light period when light equals or exceeds the photosynthetic light saturation point, I_k) can also influence GS activity in seagrasses. GS activity in belowground tissues showed little response to N_i enrichment at temperatures above or below values known for optimal growth of *Z. marina* (i.e., 25°C; Wetzel, 1982; Touchette, 1999). However, at ca. 25°C, GS activity in belowground tissues was elevated during water-column nitrate enrichment (Touchette, 1999; Fig. 3). Furthermore, removal of reduced sediments had little effect on GS activity in *Z. marina* root tissues, whereas rates of ammonium assimilation were depressed during periods of root hypoxia/anoxia (Pregnall et al., 1987). Thus, internal root anaerobiosis and anoxia would appear to be more important in controlling GS activity than external rhizosphere redox conditions. Pregnall et al. (1987) suggested that increased GS activity was associated with shorter H_{sat} and longer periods of root anoxia for *Z. marina* in deeper waters. Plants growing in such habitats may increase GS activity to attain comparable ammonium assimilation rates as plants growing in shallow (less light-limited, less anoxic) habitats.

3.4. Nitrate assimilation

The assimilation of nitrate is an energy-requiring process that is highly regulated in plants, involving activation/production of nitrate-induced proteins specifically for nitrate active transport systems (Taiz and Zeiger, 1991; Larsson, 1994). Following increased exposure to nitrate, an inducible transport system is established wherein nitrate acts as the signal to stimulate uptake. This nitrate-specific transport system is inhibited by cyanide, anaerobic conditions, and an oxidative phosphorylation uncoupler (dinitro-

Fig. 3. Model of the typical response of the enzyme glutamine synthetase (GS) to high versus low inorganic nitrogen N_i availability in *Zostera marina*. During optimal growth temperatures, GS activity increases with increasing N_i . The optimum temperature for *Z. marina* was considered as 25°C (indicated by Wetzel [1982] for *Z. marina* in southern Chesapeake Bay, and by Touchette [1999] for *Z. marina* in North Carolina coastal waters, at the southernmost extension of the range for this species on the US Atlantic Coast). As temperatures diverge in either direction from the 25°C optimum, GS response to elevated N_i decreases substantially (based on seasonal observations of *Z. marina* from Touchette, [1999]).

phenol), thus illustrating the importance of metabolic energy for its function (Taiz and Zeiger, 1991).

Although nitrate reduction can occur in roots, nitrate generally is translocated to leaves for storage in vacuoles and subsequent assimilation (known for terrestrial plants; e.g., spinach can store nitrate in excess of 4.2 mg NO_3^- g fresh weight⁻¹, levels that would be toxic to many animals; Steingrover et al., 1982). Tissue nitrate content tends to be significantly higher in above- than in belowground structures (e.g., nearly 2-fold higher in aboveground tissues of *Z. marina*; Touchette, 1999). However, in that species (perhaps indicative of N limitation), little nitrate storage apparently occurs even under water-column nitrate enrichment (0–6.8 $\mu\text{g g}^{-1}$ fresh weight in *Z. marina* under pulsed enrichment at 8 $\mu\text{M NO}_3^-$ for 14 weeks; Touchette, 1999).

Nitrate reductase (NR) is the key enzyme involved in nitrate assimilation, and the key regulatory enzyme in nitrate metabolism (Ferrario-Méry et al., 1997). The presence of nitrate induces the de novo synthesis of NR which reduces nitrate to nitrite in the cytosol, using NADH (less commonly, NADPH) as an electron donor (Beevers and Hageman, 1983). The half-life of NR is only a few hours; thus, as environmental or cellular levels of nitrate decrease, NR activity rapidly declines (Hewitt et al., 1976). In many plants, NR activity tends to follow the light curve, with highest activity during photosynthetic periods and lowest activity in darkness (Layzell, 1990).

NR appears to be tightly regulated at multiple levels. For example, light/dark modulation of NR activity involves alterations in both transcription rates and protein

phosphorylation, which increases enzyme sensitivity to Mg^{2+} inhibition (Huber et al., 1992). This mechanism, favoring light (photosynthetic) periods, allows plants to take advantage of periods when carbohydrates and reductants are most available, thereby providing the required reduction energy and carbon skeletons for nitrate assimilation (Kaiser, 1990; Turpin et al., 1991). Substrate concentration and light are considered to be the two major environmental factors that influence NR activity, but the activity of this enzyme is also affected by temperature, ammonium levels, dissolved oxygen, and carbon dioxide availability (Kenis and Trippi, 1986; de Cires et al., 1993; Müller and Janiesch, 1993). Plant physiological status (for example, carbohydrate content) is important in controlling NR response, as well (Kaiser, 1990; Berges et al., 1995).

Nitrite, the product of NR, is toxic to plant tissues (Ourry et al., 1997). To prevent accumulation, it must be further reduced to ammonium by nitrite reductase (NiR), a process that is catalyzed in plastids (typically, in chloroplasts). The reduction of nitrate to nitrite is energy-costly, requiring six electrons from the electron donor ferredoxin. The ferredoxin complex involved in nitrite reduction is the same complex used in the light reactions of photosynthesis (i.e., photosystem I; Goodwin and Mercer, 1983). Thus, there is competition for electrons between photosynthesis and nitrite reduction. Seagrasses have negligible NR activity in belowground and sheath tissues, so that nitrite reduction probably occurs mostly in leaf tissues. The process involves a specific ferredoxin-NiR (Touchette, 1999; note that terrestrial angiosperms also have a small amount of NAD(P)H-dependent NiR in proplastids of non-green tissues; Ourry et al., 1997). The potentially toxic product of nitrite reduction, ammonium, cannot be allowed to accumulate as mentioned, and must be incorporated into amino acids through the GS/GOGAT pathway as previously described.

Although in vitro NR assays (using crude extracts of fresh or frozen tissues) are in general use, sources of unknown interference have caused significant underestimates of NR activity in seagrasses. Thus, in vivo techniques (involving incubations of living tissues) are recommended (Roth and Pregall, 1988; Touchette, 1999). In vitro assays are more widely used in plant tissues because this approach limits the influence of substrate availability (e.g., electron donor NAD(P)H in V_{max} estimates) and, thus, enables assessment of maximum enzyme activity under optimal conditions. However, when considering the overall process of nitrate reduction, in vivo assays can provide valuable insights about the behavior of NR in situ (Abdin et al., 1992; Lee and Titus, 1992), including available energy for nitrate reduction and, thus, provides a valuable physiological approach.

Among the most intensively studied seagrasses for nitrate assimilation is the north temperate species, *Zostera marina*. NR in this seagrass is highly inducible and typically increases within 6 h following exposure to elevated water-column nitrate levels. Younger leaves have 2- to 5-fold higher activity than older leaves, and 10-fold higher activity than roots (Roth and Pregall, 1988; Touchette, 1999). Environmental factors such as low P_i , hypoxia, and light deprivation can significantly depress in vivo NR activity in *Z. marina* (Touchette, 1999). As in terrestrial plants, light is important in NR regulation of *Z. marina*. NR activity is lower in plants growing at depth (where light availability is reduced) relative to that of plants from shallow waters, suggesting the importance of light on overall NR response (Roth and Pregall, 1988).

Z. marina differs from many other plants in that some NR activity can be sustained throughout dark periods, provided that the surrounding water is nitrate-enriched (Gao et al., 1992; de Cires et al., 1993; Berges et al., 1995; Touchette, 1999; Fig. 4). However, the intensity and duration of NR activity is tightly coupled with, and directly related to, carbohydrate levels, wherein plants with relatively high soluble carbohydrate content tend to have much higher NR activity (Fig. 5). Therefore, if carbohydrates remain plentiful during dark periods, *Z. marina* can still assimilate and reduce nitrate at rates that are sometimes comparable to those measured in the light (Touchette, 1999). But during extended intervals of darkness or low light availability (for example, days of high turbidity following severe storm events), declines in available energy and carbohydrates coincide with a decline in NR activity (Touchette, 1999).

This apparent adaptation in *Z. marina* to assimilate water-column nitrate during dark periods (provided that adequate carbohydrate reserves are available) would provide a mechanism to enable response to pulsed nitrate enrichment whenever it occurred within a diel cycle. Nitrate is often added to coastal waters by terrestrial runoff which, in turn, is associated with storm events and increased turbidity (Kemp et al., 1983; Vitousek et al., 1997). N-Limited plants that could utilize available nitrate from the surrounding water during periods of darkness or low light would have a competitive advantage over plants that shut down nitrate assimilation in the dark. However, that not all seagrasses have inducible NR. For example, the subtropical seagrass *Halophila stipulacea* showed little inducible in vivo NR activity even following substrate additions as high as 1 mM $\text{NO}_3^- \text{N}$ for 42 h (Doddema and Howari, 1983). The low NR activity was not associated with ammonium suppression, as has been reported in other plants (Doddema et al., 1978; Doddema and Howari, 1983). It was hypothesized that *H. stipulacea* has adapted to extremely low-nitrate environments, and may have lost its ability to reduce nitrate.

3.5. Nitrogen inhibition

Udy and Dennison (1997a) proposed three categories of seagrass response to nutrient enrichment and the associated environmental conditions, paraphrased as follows: (i) growth and physiology respond favorably to the additions in low-nutrient habitats where nutrients are the only environmental factor limiting growth; (ii) there is a positive physiological response but no increase in growth, in low-nutrient habitats where environmental factors other than the added nutrient limits growth; and (iii) there is neither a growth nor a physiological response in high-nutrient environments where nutrient supplies are in excess. We propose an additional category of seagrass response, wherein (iv) there is a negative physiological response and an inhibition of growth by the added nutrient (e.g., Burkholder et al., 1992; van Katwijk et al., 1997).

It is well established that cultural eutrophication can stimulate algal overgrowth which reduces available light for seagrasses and promotes their decline (Borum, 1985; Twilley et al., 1985; Wear et al., 1999). However, recent seasonal mesocosm experiments, conducted at the southernmost extent of the range for *Zostera marina*, have documented significant lower survival of that species when grown in sandy sediments (1:3 mixture of sand to salt marsh organic muds) under water-column nitrate enrichment (pulsed daily for 8–14 weeks with $\geq 3.5 \mu\text{M NO}_3^- \text{N}$). The decreased survival that was repeatedly

Fig. 4. Model comparing nitrate reductase (NR) activity in leaves of *Zostera marina* under nitrate enrichment to the generalized NR response that has been described for many plants (algae, terrestrial angiosperms, etc.; Goodwin and Mercer, 1983; Turpin, 1991) over a diel cycle (dark bars = night). (A) When nitrate is pulsed in the light period, NR activity significantly increases. During subsequent dark periods, NR activity decreases as a typical plant response. However, *Z. marina* can maintain NR activity in the dark, provided that nitrate is still available and carbohydrate supplies are abundant. (B) When nitrate is pulsed during the dark, NR activity in *Z. marina* increases in response to the nitrate. In contrast, most plants do not respond to nitrate pulses in the dark, and NR does not increase until the next light period (based on diel observations of *Z. marina*; Touchette, 1999).

Fig. 5. Relationship between in vivo nitrate reductase (NR) activity ($\mu\text{mol nitrite per gram dry weight per hour}$) and sucrose ($\text{mg per gram fresh weight}$) in *Zostera marina* leaf tissue (from whole-plant incubations), showing a comparison of control (un-enriched, white bars) and nitrate-enriched plants (black bars; pulsed nitrate, $8 \mu\text{M NO}_3^-$). Note that plants with relatively high sucrose levels had higher NR activity. Data are given as means ± 1 S.E. ($n \geq 6$; from Touchette, 1999).

documented in experimental series was unrelated to light reduction from algal overgrowth or other source (Burkholder et al., 1992, 1994; Touchette, 1999). Treatments with water-column nitrate enrichment and light reduction in combination (from shading with neutral density screens), or with water-column nitrate enrichment and elevated temperature, led to significantly lower shoot survival (Touchette, 1999; Burkholder, 2000). This inhibitory physiological effect of nitrate enrichment was not demonstrated if the sediment, rather than the water column, was the substrate source, perhaps because of higher microbial reduction/utilization in sediments, a greater degree of diffusion-limited uptake, lower energy requirements for NH_4^+ assimilation (more abundant in sediments), and regulatory mechanisms that may be operable in the plant roots to limit nitrate absorption (Burkholder et al., 1992).

In the seasonal mesocosm experiments, the affected plants in water-column-enriched treatments showed no product feedback inhibition, that is, no ability to 'shut off' nitrate uptake (Touchette, 1999, supporting an earlier report by Roth and Pregall, 1988). Moreover, they had elevated total N and amino acid content but negligible accumulation of nitrate, nitrite or ammonium, relative to control plants without water-column nitrate enrichment (ambient nitrate at $< 2 \mu\text{M NO}_3^-$; Touchette, 1999). The data indicate that the absorbed nitrate was reduced and assimilated quickly, with limited accumulation of potentially toxic levels of ammonium or nitrite. Instead, an indirect effect of nitrate-induced internal carbon limitation was the apparent underlying mechanism (as previously shown for algae and other plants under high nitrate enrichment; physiological mechanism in Turpin, 1991; Turpin et al., 1991). Significant declines in carbohydrate levels and higher dark respiration were measured in the water-column nitrate-enriched

plants, indicating that the energy- and carbon-demanding process of nitrate uptake and assimilation to amino acids had led to an internal ‘carbon drain’ (Burkholder et al., 1992, 1994; Touchette, 1999).

Zostera marina appears to be especially sensitive to inhibition by water-column nitrate enrichment. The lack of a ‘shut-off’ mechanism for water-column nitrate uptake may have evolved—as for seagrasses that show lack of product feedback inhibition in sustained ammonium uptake—as a mechanism to enhance survival in oligotrophic, N-limited habitats (Burkholder et al., 1994). In contrast, a physiological inhibitory effect of water-column nitrate enrichment has not been documented for seagrass species *Halodule wrightii*, *Ruppia maritima*, and *Thalassia testudinum*, using a comparable range of substrate enrichment levels (Burkholder et al., 1994; Lee and Dunton, 1999). Instead, shoot production in these plants was mildly to strongly stimulated by similar enrichment levels.

Nonetheless, unusual nitrate responses among seagrass species are not restricted to *Z. marina*. Laboratory cultures of the subtropical seagrass, *Halophila decipiens*, turned brown and died within a few weeks when nitrate was the only source of N_i, whereas active growth occurred with glutamic acid or ammonium as the N source (Bird et al., 1998). As mentioned, *Halophila stipulacea* apparently is unable to use nitrate, as well (Doddema and Howari, 1983). Thus, the physiology of nitrate utilization is unusual for some seagrass species, in comparison to many other vascular plants.

A second phenomenon that has been hypothesized to occur in some seagrass species—namely, ammonia toxicity—is a common response to elevated ammonia for vascular plants. For example, research on the seagrasses *Ruppia drepanensis* and *Z. marina*, the freshwater submersed macrophyte *Potamogeton densus*, and the emergent freshwater plant *Stratiotes aloides* has revealed significant depression of growth under ammonium enrichment (Mattes and Kreeb, 1974; as described in Roelofs, 1991; Santamaría et al., 1994). This response to ammonium enrichment was unrelated to light attenuation from algal overgrowth, and ammonium toxicity was suggested as the underlying mechanism (Santamaría et al., 1994; van Katwijk et al., 1997). Sediment and water-column ammonium were high relative to levels typically found in seagrass habitats, with die-off occurring at 3–220 μM NH_4^+ in the water column, and at 500–1600 μM NH_4^+ in the sediment pore water (Santamaría et al., 1994; van Katwijk et al., 1997). Furthermore, *Z. marina* in sandy sediments was more susceptible to this apparent ammonium toxicity than plants grown in organic sediments. Elevated temperatures exacerbated the adverse response of *Z. marina* in sandy sediments to ammonium enrichment (van Katwijk et al., 1997)—similar to the conditions that had exacerbated the inhibitory effect of water-column nitrate enrichment in studies of *Z. marina* by Burkholder et al. (1992, 1994) and Touchette (1999).

4. Phosphorus physiology

4.1. P acquisition by above- and belowground tissues

As with nitrogen, seagrasses can assimilate P_i from both leaves and roots (Brix and

Lyngby, 1985). Rhizomes can also absorb P_i , but uptake rates are approximately 10-fold lower than those of roots (Brix and Lyngby, 1985). The importance of above- and belowground tissues in P uptake can vary considerably, with the relative P availability in water-column versus sediment sources as a critical determining factor. In *Ruppia maritima*, for example, P uptake by roots was not affected by P availability to leaf tissue, whereas P availability to roots strongly influenced leaf uptake (Thursby and Harlin, 1984). For plants growing in P-rich waters or sandy (P-poor) sediments, P uptake can be higher in leaves than in roots (McRoy and Barsdate, 1970; Thursby and Harlin, 1984). It has been suggested that *Zostera marina* assimilates most of its required P for growth through its leaves, and relies upon P uptake from the sediment only when P is negligible in the water column (Brix and Lyngby, 1985).

Factors such as environmental P_i levels, light, temperature, and tissue age can influence the relative importance of P_i absorption by above- versus belowground tissues (McRoy and Barsdate, 1970; Patriquin, 1972; Penhale and Thayer, 1980; Touchette and Burkholder, 1999). The rate of P uptake is strongly influenced by P availability. For example, P_i assimilation rates in *Z. marina* increase with increasing P_i in the water column and the sediment pore water (Penhale and Thayer, 1980; Pellikaan and Nienhuis, 1988). As expected, P_i uptake by belowground tissues of this seagrass was unaffected by light availability, whereas leaf P_i uptake significantly decreased during dark periods (McRoy and Barsdate, 1970; McRoy et al., 1972; Brix and Lyngby, 1985). Similarly, Pérez et al. (1994) showed that available light did not affect root P_i uptake in *Zostera noltii*, but did influence P transport to the leaves. However, P_i uptake by belowground tissues of *Thalassia testudinum* was highest during photosynthetic periods (Patriquin, 1972).

Varying proportions of the absorbed P_i can be translocated to other tissues. In one study of north temperate *Z. marina*, about 4% of the assimilated P from either leaves or roots was translocated to other tissues within 5 days (usually to younger, actively growing regions; Brix and Lyngby, 1985, in Denmark). Rates of both acropetal and basipetal translocation were depressed during dark periods, and there was no observable P excretion/leakage from above- or belowground tissues. In other research on *Z. marina* from a warmer temperature regime, Penhale and Thayer (1980) reported that from 1 to 22% of the assimilated P (tracked by the radiolabeled tracer, $[^{32}P]PO_4^{-3}$) was translocated from roots to other tissues within 12 h; and low losses (3% of the assimilated P) by excretion/leakage were detected from leaves following root uptake. In contrast to the relatively low levels of P release that were observed in that study, McRoy and Barsdate (1970) reported that more than 65% of the P_i absorbed by belowground tissues was translocated to leaves (rates $> 60 \text{ mg P m}^{-2} \text{ day}^{-1}$), followed by release to the water within 50 h.

The authors hypothesized that the apparent ‘P pump’ could represent an additional pathway in the P cycle that would allow buried [sediment] P, in both organic and inorganic forms, to re-enter the water column where it would be available to other organisms (McRoy et al., 1972). However, more recent investigations of P excretion in seagrasses indicate that P release from *Z. marina* and *Z. noltii* are strongly controlled by the relative P availability in the sediments versus the water column (Pérez and Romero, 1993). The quantity of P released was evaluated as insignificant from an ecosystem

perspective (rates from 10 to 30 nmol P g⁻¹ h⁻¹, comprising 4% or less of the total assimilated P; Pellikaan and Nienhuis, 1988; Pérez-Lloréns and Niell, 1993), although such rates could be significant in augmenting the P supplies of epiphytic community that colonized the seagrass plants (Penhale and Thayer, 1980; also see Moeller et al., 1988).

4.2. Phosphorus uptake kinetics

On the basis of sparse reports, P_i uptake affinities in seagrasses (α , range 0.12–1.10) are much lower than values reported for active ammonium uptake, but comparable to values for nitrate uptake by leaf tissues (Table 3). From laboratory incubations of *Ruppia maritima* and *Thalassia hemprichii*, $S_{\min}$ for P_i was lower for roots than for leaves, indicating that the roots could initiate P uptake at lower substrate concentrations (Thursby and Harlin, 1984; Stapel et al., 1996). The higher $S_{\min}$ in leaves may limit the period during which leaves can actively absorb P from the water column, thus minimizing the aboveground tissue contribution to the total P budget for these plants (Stapel et al., 1996). In contrast, in eutrophic environments with relatively high P levels (e.g., mean water-column PO₄⁻³P at ca. 3.5 μ M), it has been estimated that *Z. noltii* leaves can absorb 2.5-fold more of the P requirement for optimal plant growth (Pérez-Lloréns and Niell, 1995). As with P uptake affinities, maximum uptake rates ($V_{\max}$) for P_i in seagrasses (0.014–43 μ mol g⁻¹ dry weight h⁻¹) are much lower than those reported for ammonium, and comparable to rates observed for nitrate (Table 3). In addition, P_i uptake by leaves tends to be more rapid than uptake by roots, despite higher $S_{\min}$ values for leaf tissues (Thursby and Harlin, 1984; Brix and Lyngby, 1985).

As for N uptake, some seagrasses do not appear to follow the hyperbolic relationships indicative of Michaelis–Menten kinetics in P uptake. A biphasic V versus S curve was described for *Zostera noltii*, with the first phase characterized by rapid P_i uptake (first 10–20 min; up to 32 μ mol PO₄⁻³P g⁻¹ dry weight h⁻¹), followed by a substantial decline in uptake over time (typically < 6 μ mol PO₄⁻³P g⁻¹ dry weight h⁻¹; Pérez-Lloréns and Niell, 1995). The initial phase, sometimes referred to as surge uptake, is believed to represent maximum uptake by the plant, whereas the subsequent decline may indicate product feedback inhibition and/or regulation of phosphate efflux (Harrison et al., 1989; Pérez-Lloréns and Niell, 1995). The maximum P_i assimilation rates and the ‘surge’ duration may depend on the nutritional history, wherein P-starved plants would be expected to have higher uptake rates and more sustained (extended duration) uptake. For example, P_i uptake kinetics measured over 5 h in *Thalassia hemprichii* from low-P habitat (water column and sediment pore water P_i ca. 0.3 and 3.9 μ M, respectively) did not indicate a biphasic response (Stapel et al., 1996). Rather, there was a sustained period of uptake that lacked apparent product feedback inhibition over the interval tested.

4.3. Phosphorus assimilation

P_i is generally believed to be taken up by plants using phosphate transporters (Liu et al., 1998; Burleigh and Harrison, 1999; Muchhal-Umesh and Raghothama, 1999). These transporters are membrane-spanning proteins that are highly regulated among plants,

both temporally and spatially depending on the prevailing phosphorus conditions in the plant and its environment (Muchhal-Umesh and Raghothama, 1999). Unlike nitrate, the PO_4^{3-} ion does not undergo reduction prior to assimilation. It enters metabolic pathways through adenosine, wherein a phosphate ester bond is formed between P_i and ADP to produce ATP; or it bonds with hydroxyl groups of carbon chains to form simple phosphate esters (e.g., sugar phosphates; Taiz and Zeiger, 1991; Marschner, 1995). The primary process of P_i incorporation, through adenosine, occurs in mitochondria during respiration via oxidative phosphorylation, and in chloroplasts during the light reactions of photosynthesis via photophosphorylation (Taiz and Zeiger, 1991).

Because P_i is critically important in plant metabolism (Marschner, 1995), adequate supplies of P_i are necessary to ensure optimum metabolic performance. Under low P_i conditions and/or increased metabolic P_i demand, plants generally increase phosphomonoesterase activity (that is, the activities of acid- and alkaline-phosphatases or PAs). Increased PA activities enhance the ability of plants to recycle internal P_i , and to utilize P_i from environmental sources (Vincent and Crowder, 1995). These enzymes tend to be substrate-nonspecific, thereby catalyzing the release of P_i from a broad range of P-containing compounds. The ecological role of PAs, especially alkaline PA, has been linked to phosphorus deprivation in many plants (McLachlan, 1980; Burkholder and Wetzel, 1990; Pérez and Romero, 1993; Lapointe et al., 1994). In contrast, acid PA activities tend to be associated with metabolic and developmental patterns, and thus tend to be less variable (Wynne, 1977; Jansson et al., 1988). Therefore, it has been suggested that acid PAs are continually expressed and may be involved in internal phosphorus metabolism, whereas alkaline PAs, with greater external function, are more responsive to environmental P_i conditions (Vincent and Crowder, 1995).

In seagrasses, PA activities can vary depending on P_i availability, tissue age, and presence of epiphytes (Table 4). Mature leaves generally have higher PA activity than younger leaves, but the PA activity of mature leaves is believed to be commonly overestimated because of failure to remove most epiphytes while avoiding damage to the plant tissue. Some researchers have attempted to remove the epiphytic colonizers, which can have high PA activity (e.g., Burkholder and Wetzel, 1990), and have reported a trend for increased PA activity with leaf age up to early senescence (Table 4). Epiphyte accumulations can restrict carbon acquisition by macrophytes from the overlying water (Sand-Jensen, 1977), and are believed to restrict water-column P_i availability to the host plants (Moeller et al., 1988; Burkholder et al., 1990). Thick epiphytic biofilms can effectively block access to the water-column P source (Burkholder and Wetzel, 1990) and, thus, may promote enhanced PA activities in the underlying mature leaves of the host plants.

In aquatic plants under P_i limitation, PAs may be released extracellularly into the surrounding environment (Healey and Hendzel, 1979; Smith and Kalff, 1981; Lapointe et al., 1994), and/or may accumulate internally in both apoplastic and symplastic tissues (Jansson et al., 1988; Thaker et al., 1996). Alkaline PA activity has been inversely related to P_i availability in seagrasses as well as many other aquatic plants. For example, declines in alkaline PA activity were observed following PO_4^{3-} additions in *Posidonia oceanica*, and lower PA activities were observed in *Cymodocea nodosa* from P-rich waters (Pérez and Romero, 1993). However, in some studies, significant correlations

Table 4

Phosphomonoesterase (alkaline and acid phosphatase) activities reported in seagrass species including activity rates, tissue (old, young; leaf, stem, root, rhizome), and epiphyte status where available (+, with epiphytes; –, without epiphytes; and *t*, few epiphytes)

Species	Phosphatase activity ^a	Tissue	Epiphytes	Source
Alkaline phosphatase				
Temperate				
<i>Posidonia oceanica</i>	8.0 $\mu\text{mol PO}_4 \text{ g}^{-1} \text{ dw h}^{-1}$	Old leaves	–	Pérez and Romero (1993)
<i>Posidonia oceanica</i>	2.0 $\mu\text{mol PO}_4 \text{ g}^{-1} \text{ dw h}^{-1}$	Young leaves	<i>t</i>	Pérez and Romero (1993)
<i>Posidonia oceanica</i>	1–3 $\mu\text{mol PO}_4 \text{ g}^{-1} \text{ dw h}^{-1}$	Roots		Pérez and Romero (1993)
<i>Posidonia oceanica</i>	6.0 $\mu\text{mol pNP}^* \text{ g}^{-1} \text{ dw h}^{-1}$	Leaf		Hernández et al. (1994a)
<i>Zostera marina</i>	4.6 $\mu\text{mol pNP g}^{-1} \text{ dw h}^{-1}$	Leaf		Hernández et al. (1994a)
<i>Zostera marina</i>	0.3–0.8 $\mu\text{mol pNP g}^{-1} \text{ fw h}^{-1}$	Leaf	–	Touchette (1999)
<i>Zostera marina</i>	0.1–1.5 $\mu\text{mol pNP g}^{-1} \text{ fw h}^{-1}$	Root-rhizome		Touchette (1999)
<i>Zostera noltii</i>	10 $\mu\text{mol pNP g}^{-1} \text{ dw h}^{-1}$	Leaf		Hernández et al. (1994a)
<i>Zostera noltii</i>	19 $\mu\text{mol pNP g}^{-1} \text{ dw h}^{-1}$	Young leaf		Hernández et al. (1994b)
<i>Zostera noltii</i>	15 $\mu\text{mol pNP g}^{-1} \text{ dw h}^{-1}$	Old leaf		Hernández et al. (1994b)
<i>Zostera noltii</i>	1.6 $\mu\text{mol pNP g}^{-1} \text{ dw h}^{-1}$	Stem		Hernández et al. (1994a)
<i>Zostera noltii</i>	1.8 $\mu\text{mol pNP g}^{-1} \text{ dw h}^{-1}$	Roots		Hernández et al. (1994a)
Tropical/subtropical				
<i>Cymodocea nodosa</i>	8–18 $\mu\text{mol PO}_4 \text{ g}^{-1} \text{ dw h}^{-1}$	Old leaf	+	Pérez and Romero (1993)
<i>Cymodocea nodosa</i>	4–8 $\mu\text{mol PO}_4 \text{ g}^{-1} \text{ dw h}^{-1}$	Young leaf	+	Pérez and Romero (1993)
<i>Cymodocea nodosa</i>	1–5 $\mu\text{mol PO}_4 \text{ g}^{-1} \text{ dw h}^{-1}$	Roots		Pérez and Romero (1993)
<i>Thalassia testudinum</i>	< 35 $\mu\text{M PO}_4 \text{ g}^{-1} \text{ dw h}^{-1}$	Apical leaf	+	Lapointe et al. (1994)
<i>Thalassia testudinum</i>	< 100 $\mu\text{M PO}_4 \text{ g}^{-1} \text{ dw h}^{-1}$	Distal leaf	+	Lapointe et al. (1994)
Acid phosphatase				
Temperate				
<i>Zostera marina</i>	17 $\Delta \text{ Ext g}^{-1} \text{ fw min}^{-1}$	Leaf		Lutova and Feldman (1981)
<i>Zostera marina</i>	4–8 $\mu\text{mol pNP g}^{-1} \text{ fw h}^{-1}$	Leaf	–	Touchette (1999)
<i>Zostera marina</i>	1–13 $\mu\text{mol pNP g}^{-1} \text{ fw h}^{-1}$	Root-rhizome		Touchette (1999)
<i>Zostera noltii</i>	46 $\Delta \text{ Ext g}^{-1} \text{ fw min}^{-1}$	Leaf		Lutova and Feldman (1981)

^a pNP designates the in vitro assay product, *p*-nitrophenyl.

between seagrass alkaline PA activity and low P_i have been lacking (e.g., Hernández et al., 1994a; Touchette and Burkholder, 1999), possibly because growth was not P-limited, or because other environmental and developmental variables more strongly influenced alkaline PA activities.

Factors such as light, temperature, season, pH, salinity, and epiphytic development influence seagrass alkaline PA activity (Pérez and Romero, 1993; Hernández et al., 1994a,b; Lapointe et al., 1994; Touchette and Burkholder, 1999). Under non- P_i limited conditions, seagrass alkaline PA activities can be influenced, as well, by ambient metabolic conditions (e.g., *Z. marina*; Touchette and Burkholder, 1999). Fluctuations in alkaline PA activity may be important in optimizing P_i availability for metabolic processes involved in carbon and nitrogen metabolism (see below). For example, in belowground tissues of *Z. marina* when soluble carbohydrate levels were low, alkaline PA significantly increased; and as sucrose demand increased in belowground tissues (indicated by higher sucrose synthase activity, which is associated with higher carbon

demand), alkaline PA activities increased (Touchette, 1999; Touchette and Burkholder, 1999). Both responses illustrate the importance of P_i supply in regulation and/or activation of carbohydrate metabolic processes.

Although acid PAs in terrestrial plants can be involved in important functions such as seed germination, secondary thickening of cell walls, and carbohydrate metabolism (Basra et al., 1989; Thaker et al., 1996), little is known about acid PAs and seagrasses (Table 4). In vitro acid PA activities have been used as an indicator of thermostability in *Zostera marina* and *Z. noltii* (Lutova and Feldman, 1981). *Z. noltii* appeared to tolerate higher temperatures than *Z. marina* based on enzyme activity. This information is supported by the geographic distribution and habitat preferences of the two species.

Acid PA activities have also been used as an indicator of recognition and acceptance of pollen fertilization in the seagrasses, *Enhalus acoroides*, *Thalassia hemprichii* and *Halophila stipulacea* (Pettitt, 1980). Following acceptance of pollen, an enzymatic system involving acid PA is activated to digest the cuticular layer of the stigma for entry of the pollen tube (Pettitt, 1980). In the intine of mature pollen from *T. hemprichii*, acid PA activity was mostly localized within cytoplasmic inclusions; and acid PA activity was detected, as well, in stigma papillae tips of all three examined seagrass species (Pettitt, 1980). In *Zostera marina*, acid PA has been linked to carbon and nitrogen metabolism, wherein increases in acid PA activity were associated with increases in the carbon demand of belowground tissues (Touchette and Burkholder, 1999). In leaf tissues, increased acid PA activity was also associated with decreases in both sucrose levels and GS activity.

5. Carbon, nitrogen, and phosphorus ratios

With increasing interest in the nutritional ecology of seagrasses, researchers have begun to consider carbon, nitrogen and phosphorus content, or ratios of these macronutrients in seagrass tissues, as indicative of nutrient status. This concept is based on Redfield et al. (1963) for oceanic seston, wherein the C:N:P ratio in phytoplankton was reported at ca. 106:16:1 (weight basis) under optimal/non-limiting growth conditions. Deviation from this ratio would indicate potential nutrient limitation. The concept had earlier been applied to freshwater macrophytes by Gerloff (1963, 1969), modified to account for the substantially higher proportion of carbon-containing structural tissues in macrophytes than in phytoplankton. Unfortunately, early attempts to compare C:N:P tissue content in seagrasses failed to provide generalizations about seagrass nutritional status because of their seemingly aberrant (high) carbon content, relative to Redfield's stoichiometry (Atkinson and Smith, 1984). Tissue nutrient content was also found to vary with species, tissue type, season, age, and environmental conditions (Pérez-Lloréns and Niell, 1993; Hemminga et al., 1995). For example, tissue C:N ratios in intertidal *Z. noltii* were reported as highest in summer and lowest in winter, with higher values in below- than in aboveground tissues (Pérez-Lloréns and Niell, 1993).

Duarte (1990) later demonstrated that the previously considered inability to use C:N:P values as indicative of nutritional status in seagrasses stemmed from both a limited

dataset and the inability to compare specific data with values known to represent nutrient-balanced growth. Following extensive compilation of data available for 27 seagrass species of varying geographic distribution, Duarte (1990) reported C:N:P ratios (means $\pm$ 1 S.E., as percent dry weight) of $33.6 \pm 0.31\%$ C, $1.92 \pm 0.05\%$ N, and as $0.23 \pm 0.11\%$ P. Despite high intra- as well as interspecific variability, the distribution curves for all species considered collectively were skewed to the left for N and P. Since N and P values were not normally distributed, median values (1.82% N and 0.2% P) were used to estimate an overall C:N:P ratio of 474:21:1 for the seagrasses considered. This ratio is clearly lower than the average C:P and C:N ratios typically reported for a range of seagrasses, as well as for kelps and other macroalgae with high structural carbon (550 and 30, respectively; Atkinson and Smith, 1984), and higher than the ratio reported by Redfield (1963; phytoplankton). From these data, Duarte (1990) inferred that C-limited growth is rare in seagrasses relative to N- or P-limited growth; and that higher C:N and C:P values in seagrass tissues reflect higher structural C content relative to that of phytoplankton.

Spatial and temporal variations in C:N:P ratios have since been used, with some success, as indicators of seagrass nutritional status and in tracking nutrient supply sources, especially N (e.g., Fourqurean et al. 1992b, 1997). They can be especially helpful in assessment of nutrient-enriched seagrasses. However, because factors other than nutrients can limit seagrass growth, inference of degree of nutrient limitation from such data becomes more difficult. Moreover, under field conditions the time of year as well as intra- and inter-species differences—which can be substantial (Agawin et al., 1996; Fourqurean et al., 1997)—need to be carefully considered in interpreting tissue nutrient content and other physiological variables (Agawin et al., 1996; Udy and Dennison, 1997a,b).

6. N and P interactions with carbon metabolism

6.1. Carbon and nitrogen

Many physiological and biochemical processes are interconnected. Therefore, an alteration in one process is likely to cause cascade responses affecting other biochemical mechanisms. This is especially true for carbon and nitrogen metabolism, wherein C and N pathways must compete for assimilatory energy (reduced ferredoxin, NADPH, and ATP; Turpin, 1991; Turpin et al., 1991). Thus, as N uptake and assimilation increase—especially involving nitrate—competition for photosynthates develops among various metabolic pathways (Marschner, 1995; Ferrario-Méry et al., 1997; Fig. 1).

The assimilation and reduction of nitrate is one of the most energy-costly processes in plants, and includes the high-energy costs of nitrate reduction as well as the cost of carbon skeletons needed for formation of amino acids (Foyer et al., 1994; Ferrario-Méry et al., 1997). This cost is indicated from respiratory responses; it has been estimated that leaves can expend as much as 25% of their total respiratory energy on N_i assimilation, alone (Bloom et al. 1989, 1992; Wojcieszka-Wyskupajtyś, 1996). Such high costs of nitrate assimilation—a biochemical process that is conserved among many plants—

Fig. 6. Respiration of *Zostera marina* leaves (from whole-plant incubations) following low-level nitrate additions (0–10 $\mu\text{M NO}_3^-$). Note that respiration increased with increasing nitrate enrichment. Data are given as means $\pm$ 1 S.E. ($n = 2$; from Touchette, 1999).

appear to be operable in seagrasses. For example, leaf respiration rates in *Zostera marina* nearly doubled when plants were exposed to low-level water-column nitrate enrichment (up to 10 $\mu\text{M NO}_3^-$ in 8-h experiments; Fig. 6; Touchette, 1999).

As mentioned, the relative intensity of NR activity observed in the seagrass, *Zostera marina* during dark periods has been shown to depend largely on the levels of soluble carbohydrates available in the leaves (Touchette, 1999; also shown for barley by Aslam and Huffaker, 1984; Fig. 5). In this seagrass, which is believed to have evolved in N-limited waters, increased NR activity in response to nitrate pulses in light or darkness would have been favored over the strict uptake modulation in the light that has been observed in many terrestrial and aquatic species (Kenis and Trippi, 1986; Gao et al., 1992; Huber et al., 1992). The ability to reduce nitrate regardless of time of day would allow *Z. marina* to take advantage of infrequent, short-term night pulses of nitrate and/or pulses associated with light-attenuating storm events (Roth and Pregnall, 1988; Burkholder et al., 1994). Such response would require redirection of carbohydrates from storage reserves to production of the energy and carbon skeletons needed to reduce nitrate and form amino acids (Turpin, 1991; Ferrario-Méry et al., 1997).

However, if high water-column nitrate levels persisted (as characteristic of many present-day coastal waters under cultural eutrophication), without a product feedback mechanism to prevent further uptake (Roth and Pregnall, 1988; Touchette, 1999), these plants would eventually deplete available carbohydrates to levels where growth was substantially compromised (Burkholder et al., 1992). Recent research documented depressed productivity and survival, and decreased soluble carbohydrate levels (ca. 1.4-fold lower than in un-enriched controls) in *Z. marina* under low levels of water-column nitrate enrichment (pulsed daily at 3.5–30.0 $\mu\text{M NO}_3^-$ for 8–14 weeks; decreases unrelated to light reduction from algal-induced or other turbidity—Burkholder

et al., 1992; Burkholder et al., 1994; Touchette, 1999). Thus, for seagrasses such as *Z. marina* that undergo periods of carbon-limited growth, sustained increases in water-column nitrate loading could further limit productivity (Burkholder et al., 1992).

6.2. Carbon and phosphorus

Internal phosphorus conditions can strongly influence carbon metabolism (Touchette, 1999; Fig. 1). Phosphorus is important in metabolic energy transfer (for example, ATP, NAD(P)H); in forming intermediates of metabolic pathways (e.g., starch and sucrose synthesis/degradation intermediates glucose 6-phosphate and fructose 1,6-bisphosphate); and in regulating enzyme activity through protein phosphorylation/dephosphorylation (e.g., NR and enzymes involved in carbon metabolic pathways such as sucrose-P synthase and phosphoenol pyruvate carboxylase; Marschner, 1995; MacKintosh, 1997). Protein phosphatases and protein kinases function in regulating cell division, polysaccharide synthesis, cell wall development, and nitrate reduction/assimilation (Walker, 1999). P_i is also required to facilitate the exchange of P_i from the cytosol, and of triose phosphates from the chloroplast, via the phosphate translocator (Marschner, 1995). During this process, triose-P that enters the cytosol may be used to synthesize more complex carbohydrates. Thus, cytosolic P_i is necessary for the release of triose-P from the chloroplast and its utilization in the formation of complex soluble polysaccharides. When P_i becomes relatively low in the cytosol, triose-P may accumulate in the chloroplast, thereby enhancing starch production (Marschner, 1995).

Under environmental P_i limitation, plant photosynthesis can be depressed by as much as 50% (Dietz and Foyer, 1986; Rao et al., 1989; Usuda and Shimogawara, 1991), possibly as a result of lower Rubisco activation and/or decreased recycling of ribulose 1,5-bisphosphate (Brooks, 1986; Rao et al., 1989). Lower levels of internal P_i may also develop internally following accumulation of phosphorylated intermediates of starch and sucrose synthesis/degradation (e.g., glucose 6-phosphate and fructose 1,6-bisphosphate; Sharkey, 1985; Rao et al., 1989). In *Zostera marina* under non-limiting P_i conditions, fluctuations in PA activities appear to be important in optimizing C and N metabolism (Touchette and Burkholder, 1999). For example, in belowground tissues of *Z. marina* when sucrose levels were low, alkaline PA activity increased; and as sucrolysis increased, the activity of both acid and alkaline PA significantly increased as well. Thus, during periods of increased carbon mobilization/utilization in this seagrass—for example, under elevated temperatures or increased N assimilation— P_i demands would be expected to increase (Touchette and Burkholder, 1999).

7. Future research directions

Seagrasses have evolved to tolerate high-salinity submersed habitats that are characteristically unsuitable for most angiosperms. The necessary adaptations for survival and growth in such environments undoubtedly have altered many physiological processes in these plants, including processes involved in nutrient acquisition and response to changing nutrient gradients.

Most of the available information about seagrass nutritional physiology to date has been obtained from study of only a few species. The basic nutritional characteristics of many seagrass species remain to be determined and compared across geographic regions. The species that, to date, have been examined in some detail have shown considerable variation in nutritional response. Thus, inferences about a well-studied species should not be applied a priori to that species in other geographic regions, or to other seagrasses.

Recent gains in general understanding about the physiological responses of seagrass species to nutrient gradients have shown promise in application to management efforts to strengthen protection of seagrass meadows. For example, physiological variables such as tissue C:N:P content have begun to be developed as indicators of nutrient conditions and anthropogenic nutrient enrichment. Seagrass tissue nutrient content effectively ‘integrates’ nutrient pulses, and can indicate a pulsed enrichment event that typical water quality sampling—if not conducted with coincident timing—could easily miss. Additional physiological variables (for example, NR activity) should be rigorously examined for potential utility as indicators of seagrass nutritional status. Such variables would be expected to be species-specific, and likely would not be applicable across extended regions. However, physiological indices, together with data on shoot densities and growth, would provide valuable information in tracking the health of local seagrass populations under cultural eutrophication, which is increasing in many coastal areas because of exponential human population growth and development of coastlands (Vitousek et al., 1997).

Greater emphasis is needed on research to assess the roles of factor interactions—among inorganic and organic N, P, C; and among nutrients and temperature, light, and flushing rate—in controlling seagrass physiology and growth. The influence of interactions between nutrients and other trophic components (dominant algae, herbivores and predators) on the physiology as well as the ecology of many seagrass species should also be examined (e.g., Williams and Ruckelshaus, 1993; Neckles et al., 1993). Finally, the chronic impacts of low to moderate nutrient enrichment on seagrass populations merit strengthened focus. In this writing we described a subtle but serious interaction between $N \times C$ in adversely affecting *Zostera marina* at the southernmost extension of its range—namely, the internal ‘carbon drain’ which can be imposed by sustained nitrate uptake. A similar effect was documented in algae a decade ago (Turpin, 1991; Turpin et al., 1991), and in oligotrophic pine species (Aber, 1992). Additional chronic impacts of nutrient enrichment, both beneficial and detrimental, likely affect seagrass species. Experiments are needed to assess chronic impacts of N and P enrichment on other seagrasses, extended over the growing season(s) for the species; and to elucidate the underlying physiological mechanisms that ultimately control the survival, or the loss, of these ecologically important marine angiosperms under the increasing nutrient regimes that have come to characterize many coastal waters.

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