1   Title: Seagrass restoration reestablishes the coastal nitrogen filter through enhanced burial

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14   **Abstract**

15   Seagrass meadows perform an important ecological function as filters for incoming nutrients

16   from surrounding watersheds, especially nitrogen (N). By enhancing N removal processes,

17   including N burial in sediments and denitrification, seagrass meadows improve water quality.

18   With accelerating losses of seagrass meadows worldwide, seagrass restoration plays a key role in

19   reestablishing these coastal ecosystem functions. However, few measurements exist of N burial

20   rates in temperate seagrass meadows and none have been published for restored meadows. In this

21   study, we measured N burial rates in a large (6.9 km^2) restored eelgrass (*Zostera marina*)

22   meadow and compared N removal through burial to previous measurements of removal via

23   denitrification. We also compared N removal to inputs from external loading and fixation and to

24   N assimilation in seagrass biomass. We found that, in this meadow, burial was the dominant

25   process of N removal; the burial rate of 3.52 g N m^{-2} y^{-1} was comparable to rates in natural

26   meadows within 10 years after seeding and was more than 20x the rate in adjacent bare

27   sediments (0.17 g N m^{-2} y^{-1}). We also found that the high rates of N assimilation (2.62 g N m^{-2} y^{-1}

28   ) created a substantial though temporary sink for nitrogen during the growing season. Our

29   results highlight how seagrass meadows mediate N cycling through high rates of burial, which to

30   date has been understudied in the literature. The successful return of the N filter function after

31   restoration, shown here for the first time, can motivate continued efforts for seagrass restoration

32   and conservation.

33

34   **Introduction**

35   Seagrass meadows are highly productive ecosystems that provide a wide array of

36   ecosystem services, ranging from supporting tourism and recreation to regulating climate via

37   carbon sequestration (Barbier et al. 2011). Through their high productivity, seagrass can strongly

38   influence the nitrogen (N) cycle in coastal waters. Large amounts of N are assimilated into

39   seagrass biomass, which turns over slowly compared to algal biomass, leading to temporary

40   retention of nitrogen (on the order of weeks to months); retention of N in seagrass reduces N

41   availability to ephemeral algae species (Banta et al. 2004; McGlathery et al. 2007). Seagrass

42   meadows can also remove nitrogen from coastal waters by enhancing two removal processes,

43   burial and denitrification, in seagrass sediments. Seagrass increase N burial both by the physical

44   processes of increased sedimentation (due to wave and current attenuation by the seagrass

45   canopy) and sediment stabilization by roots and rhizomes (van der Heide et al. 2011; Hansen and

46   Reidenbach 2012), and by the biological process of accumulation of recalcitrant seagrass tissue

47   in the sediment (Middelburg et al. 2004). N can remain buried in seagrass sediments over long

48   time scales (from decades to centuries) (Mateo et al. 1997). Seagrass roots also exude oxygen

49   and labile carbon into the sediment, which can stimulate coupled nitrification-denitrification,

50   leading to permanent N removal through the microbial transformation of biologically available

51   nitrate into inert dinitrogen gas (Iizumi et al. 1980; Aoki and McGlathery 2018). Taken together,

52   these retention and removal processes form a 'filter' for nitrogen that increases coastal water

53   quality and slows the movement of nitrogen inputs from watersheds to the open ocean.

54   The seagrass filter function promotes a positive feedback for seagrass growth. By

55   reducing particulates in the water column (i.e. through wave attenuation and sediment

56   stabilization), the presence of the meadow can increase light availability deeper in the water

57   column, supporting greater seagrass growth (Carr et al. 2010). In addition, uptake of dissolved N

58   into seagrass biomass limits N availability for algal growth that can shade the seagrass (Gurbisz

59   et al. 2017). This positive feedback highlights the role of seagrass as an ecosystem engineer that

60   modifies its habitat to better suit its needs, creating a stabilizing effect that allows the seagrass

61   ecosystem to flourish. The restoration of the N filter function to levels observed in natural

62   systems may therefore be a useful measure of the overall success of a seagrass restoration.

63   While some N cycling processes in seagrass meadows have been widely studied, our

64   understanding of N removal as an ecosystem function remains incomplete. Previous

65   measurements of N removal in seagrass meadows have focused primarily on denitrification.

66   Recent studies have generally found elevated rates of denitrification in seagrass meadows

67   compared to other estuarine habitats (Eyre et al. 2011, 2013; Smyth et al. 2013), but in some

68   cases, rates were equivalent between seagrass and unvegetated sediments (Russell et al. 2016;

69   Zarnoch et al. 2017). In contrast, measurements of N burial in seagrass meadows are few and

70   focus mainly on peat-forming *Posidonia* species (Romero et al. 1994; Mateo and Romero 1997;

71   Gacia et al. 2002, see Table 1). However, a recent study by Eyre et al. (2016) found elevated

72   rates of both denitrification and N burial within *Zostera* seagrass communities relative to subtidal

73   flats in three sub-tropical lagoons; the seagrass community N removal rates contributed

74   significantly to lagoon-wide N removal. Further work is needed to better characterize N burial in

75   temperate seagrass meadows and to assess the relative contributions of burial and denitrification

76   to net N removal.

77   Retention in seagrass biomass, the temporary portion of the N filter function, is better

78   studied than N removal and is generally found to be a dominant flux of nitrogen in seagrass

79   meadows (McGlathery 2008). In non-eutrophic estuarine systems, N demand typically exceeds

80   N loading (Pedersen et al. 2004), indicating that most N entering these systems passes through

81   the primary producer pool (McGlathery et al. 2007). Assimilation rates typically peak during the

82   summer growing season in temperate meadows (Pedersen and Borum 1993; Risgaard-Petersen et

83   al. 1998; Park et al. 2013). Seagrass tissue is more recalcitrant than algal biomass (Banta et al.

84   2004), indicating that the temporary N retention during the growing season reduces N

85   availability compared to algae-dominated systems, thus contributing to the N filter and

86   supporting the positive feedback for seagrass growth. Given that N assimilation fluxes are large

87   relative to biogeochemical N processes, the N retention component of the N filter function is

88   significant, despite the short-term impact. Comparing the relative importance of N retention in

89   seagrass biomass to N removal through burial and denitrification will clarify the different

90   ecological dimensions of the seagrass N filter function.

91   The coastal N filter function is lost when seagrass meadows decline due to anthropogenic

92   impacts, including dredging, eutrophication, and marine heatwaves. Globally, seagrass loss is

93   accelerating (Orth et al. 2006a, Waycott et al. 2009). Habitat restoration reestablishes natural

94   meadow processes (Orth and McGlathery 2012; Orth et al. 2012), including carbon sequestration

95   at rates on par with natural, undisturbed meadows (Greiner et al. 2013, Oreska et al. 2017a). We

96   expect that restoration also reestablishes the coastal N filter function of seagrass meadows to a

97   level comparable to natural meadows; however, this effect has yet to be examined.

98   The purpose of this study was to assess the return of the coastal nitrogen filter in a

99   restored *Zostera marina* (eelgrass) meadow located in a shallow coastal lagoon in Virginia,

100   USA, and to compare the magnitude of N filtering processes to both external N loading from

101   watershed sources and to internal N loading from N fixation within the meadow. Historically,

102   eelgrass dominated the Virginia coastal bays but a combination of wasting disease and the

103 impacts of a hurricane in 1933 lead to local extinction (Orth and McGlathery 2012). Restoration

104 by seeding was begun in 2001 by the Virginia Institute of Marine Sciences (VIMS) leading to the

105 establishment of >25 km^2 of mature eelgrass meadows (http://web.vims.edu/bio/sav/). This

106 restoration provides a unique opportunity to assess the impact of seagrass presence on retention

107 and removal of nitrogen in the lagoon. By comparing measurements made in the mature restored

108 meadow to measurements in adjacent, unrestored ("bare") sediments, we were able to directly

109 assess the nitrogen cycle in the lagoon with and without seagrass. In previous studies, we have

110 measured the effect of this seagrass restoration on biogeochemical fluxes, including N fixation

111 and denitrification (Aoki and McGlathery 2018, 2019). Here, we collected detailed

112 measurements of N burial and N assimilation and compared these components to N fixation,

113 denitrification, and external N loading rates, in order to understand how restoration affected the

114 coastal N filter.

115 **Methods**

116 ***Study site***

117   This study was conducted at the restored *Z. marina* meadow in South Bay, a shallow

118 lagoon located on the Eastern Shore of Virginia, along the USA mid-Atlantic coast (37.269852, -

119 75.810716). The South Bay meadow is one of four restored seagrass meadows in Virginia that

120 were seeded as part of a landscape-scale restoration project (Orth et al. 2012). In South Bay,

121 seeds were broadcast in 0.4 ha plots in 2001; these original seed plots eventually coalesced into a

122 contiguous meadow that has continued to spread (Figure 1). In 2015, the restored meadow was

123 approximately 6.9 km^2 (http://web.vims.edu/bio/sav/).

124 ***Nitrogen cycle processes***

125   To assess the magnitude of the coastal filter in the restored seagrass meadow, we

126   compared N inputs to N removal and retention measured in the meadow and in adjacent bare

127   sediments. N inputs included loading from terrestrial and atmospheric sources and N fixation

128   occurring within the meadow, including the contributions from sediment N fixation and

129   epiphytic N fixation occurring on seagrass leaves (potentially an important component of total N

130   fixation, Moriarty and O'Donohue 1993; Cole and McGlathery 2012). N removal consisted of

131   burial and denitrification, and N retention was estimated by measuring N assimilation in seagrass

132   biomass. Burial and assimilation rates were measured directly in this study, and fixation and

133   denitrification data were compiled from recent studies in the South Bay meadow (Aoki and

134   McGlathery 2018, 2019). N loading data were adapted from a nitrogen budget prepared by

135   Anderson et al. (2010) for a neighboring coastal lagoon with similar watershed land use.

136   Fixation, denitrification, and assimilation were measured seasonally in spring, summer, and fall;

137   in order to determine annual rates, winter rates were estimated as half of the rates during fall,

138   based on ratios of fall and winter measurements in other *Zostera* systems (Risgaard-Petersen et

139   al. 1998; Eyre et al. 2011) and low winter shoot densities in this system (unpublished data). The

140   annual rates were compared in units of g N m^{-2} y^{-1}; standard errors and degrees of freedom for

141   each rate were propagated using the approach and equations described by Lehrter & Cebrian

142   (2010) in order to calculate confidence intervals for each rate. Bare measurements of N burial

143   were made in sediments outside of the established seagrass meadow, approximately 150 m from

144   the meadow edge. For denitrification and fixation, we relied on rates reported in earlier studies

145   from this site (Aoki and McGlathery 2018, 2019). As reported in those studies, bare

146   measurements of denitrification and N fixation were made in experimentally cleared sub-plots (4

147   m^2) in the meadow interior in order to isolate the direct effects of seagrass growth, i.e. oxygen

148   and carbon exudation, and to eliminate the effects of confounding factors outside the meadow,

149   such as varying water depths and flow regimes, on sediment biogeochemistry.

150   *N loading*

151   Inputs of nitrogen to South Bay from terrestrial and atmospheric sources were assessed

152   based on an earlier assessment of nitrogen cycling in Hog Island Bay, a coastal lagoon located

153   approximately 20 km north of South Bay, with a similar lagoon area, ratio of watershed area to

154   lagoon area, and level of development and land use in the watershed (Anderson et al. 2010).

155   Terrestrial and atmospheric inputs to Hog Island Bay were therefore considered to be broadly

156   similar to South Bay (Cole 2011). The nitrogen sources evaluated in the Anderson study were

157   base flow, surface water run-off, groundwater discharge, and atmospheric deposition. Base flow

158   was calculated from stream discharge and nitrate concentrations; N loading from run-off and

159   groundwater discharge were estimated as 40% and 33% of base flow loading respectively, based

160   on a combination of direct measurements and modeling (see Anderson et al. 2010 for details).

161   Atmospheric deposition (wet and dry) was calculated from samples collected at a meteorological

162   tower located on Hog Island, the barrier island adjacent to Hog Island Bay, from 1990-1999, and

163   corrected for sample preservation error. For the South Bay meadow budget, the loading rates

164   determined in the Anderson study (kg N ha^{-1} y^{-1}) were scaled based on the area of the South Bay

165   lagoon and watershed, as determined by Hayden & Porter (2001).

166   *N fixation and denitrification*

167   Daily rates of N fixation and denitrification were calculated from hourly rates previously

168   measured in this system, and full details of the incubation methods are available elsewhere (Aoki

169   and McGlathery 2018 and Aoki and McGlathery 2019). Briefly, hourly acetylene reduction rates

170   in sediments and by epiphytes on seagrass blades were measured from June 2016-August 2017

171 using jar incubations; rates were converted to N fixation rates using the theoretical 3:1 ratio

172 (Seitzinger and Garber 1987; Welsh 2000). To scale hourly N fixation rates to annual rates, dark

173 and light incubation rates were pooled, as there was no significant effect of light on either

174 sediment or epiphyte N fixation (see Aoki and McGlathery 2019), and the pooled rates were

175 integrated over 24 hours to calculate daily rates for each season. Total daily N fixation was

176 calculated as the sum of sediment and epiphyte rates; epiphyte N fixation was a maximum of

177 20% of total N fixation in summer. Daily seasonal rates were then integrated over each season to

178 calculate annual rates.

179 Hourly denitrification rates in sediments were measured from June 2014-July 2015 using

180 an in situ, push-pull, isotope pairing method; the method is described in detail in Aoki and

181 McGlathery (2017). Using the push-pull method, the hourly rate measurements were made under

182 variable in situ daylight conditions; extrapolating from hourly to daily rates therefore required

183 assumptions about sediment denitrification under dark conditions. Under light conditions, the

184 denitrification rates were characterized by "hotspots" where maximum rates exceeded median

185 rates by an order of magnitude. We attributed these hotspot rates to the effects of seagrass root

186 exudation (see Aoki and McGlathery 2018), and assumed the hotspots would not occur under

187 dark conditions. We therefore calculated a dark rate based on the median of the light rates

188 excluding the hotspot rates, and extrapolated to daily rates by scaling the light and dark rates for

189 12 hours each. There is some evidence that denitrification rates in seagrass meadow sediments

190 may be greater under dark conditions compared to light conditions, due to the lack of

191 competition for nitrate from autotrophs (Welsh et al. 2000; Eyre et al. 2011, 2013). The daily

192 rates reported here may therefore underestimate daily denitrification. Fall and spring rates of

193 denitrification in the bare sediments were estimated based on the ratio of bare rates to seagrass

194   rates during summer. Daily seasonal rates were integrated by each season to calculate annual

195   rates.

196   *Burial*

197   N burial rates in seagrass sediments and bare sediments were calculated from sediment

198   accretion rates and the N content of the sediment. Accretion rates (cm yr^{-1}) were provided in

199   previous work at the site by Greiner et al. (2013) that used ^{210}Pb dating of sediment cores to a 20-

200   cm depth; the accretion rates were confirmed in a later study by Oreska et al. (2017c). To

201   determine N content, small sediment cores (2.5 cm diameter, 6 cm depth) were collected in 2013

202   from 16 sites distributed across the meadow and 2 bare sediment sites outside the meadow, with

203   4 replicate cores per site. An additional 6 sites clustered in the interior of the meadow and an

204   additional bare site outside the meadow were sampled to a depth of 5 cm in 2014, with 5

205   replicate cores per site. A total of 22 seagrass sites and 3 bare sites were analyzed for N content.

206   Sediments were dried to a constant weight at 60ºC, and bulk density was calculated from the dry

207   weight and wet volume of the sample. N content (%N) was measured using a Carlo Erba NA

208   2500 Elemental Analyzer, bulk N content (mg cm^{-3}) was calculated from %N and bulk density,

209   and N burial rates (g N m^{-2} y^{-1}) were calculated from bulk N content and accretion rates.

210   As the South Bay meadow has expanded from the original seed plots, different areas of

211   the meadow have been restored for varying amounts of time, i.e., the meadow age varies

212   spatially. Long-term monitoring has shown a 5-year lag between the initial seeding and changes

213   to sediment characteristics and has shown that after 9 years, shoot densities in the meadow

214   stabilized around 500 shoots m^{-2} (McGlathery et al. 2012). The accretion rates measured by

215   Greiner et al. (2013) showed the same pattern of a slight increase in accretion over the first five

216   years of restoration, followed by a more rapid increase over years 5-9. We therefore calculated

217 separate burial rates for three age brackets of restored meadow: 1-5 years, 5-9 years, and >9

218 years. Aerial photographs from an annual survey by VIMS (http://web.vims.edu/bio/sav/)

219 allowed us to determine the age of the 22 plots sampled for burial within the meadow and to

220 calculate mean N content and burial rates for each age bracket. We also used the aerial

221 photography (digitized in ArcGIS) to determine the extent of meadow coverage within each age

222 bracket in 2015 in order to calculate a weighted average burial rate for the entire meadow.

223 *Assimilation*

224   Assimilation of N into seagrass biomass was calculated from seagrass productivity rates

225 and seagrass tissue N content. Productivity was measured using the leaf-marking technique

226 (Short and Duarte 2001) in June, August, and October 2016 and in April and June 2017.

227 Replicate 20 x 10 cm wire frames were anchored in the seagrass sediment, and all shoots within

228 the frame were marked by puncturing the sheath bundle with a 25.5-gauge needle. After 10-15

229 days, the shoots, including rhizomes, were carefully harvested. Each shoot was separated into

230 new growth (unmarked new leaves and leaf tissue below the needle scar) and old growth (tissue

231 above the scar); new and old aboveground tissue was dried to a constant weight to determine

232 shoot specific growth rates over the marking period (g shoot^{-1} d^{-1}). The plastochrone interval, or

233 the time period between the production of each consecutive leaf, was calculated based on the

234 number of new leaves that appeared during the marking period. The total rhizome length and

235 average internode length were measured and rhizomes were also dried to a constant weight in

236 order to calculate a length-to-weight ratio for belowground biomass. Because *Zostera marina*

237 produces a new rhizome node for each new leaf, the belowground biomass growth rates (g shoot^{-}

238 ^{1} d^{-1}) could be calculated from the average internode length, length-to-weight ratio, and the

239 plastochrone interval. Four productivity frames were deployed at each of 3 plots in each

240   sampling month except in June when frames were deployed at 6 plots; on average, 68 marked

241   shoots were recovered during each marking period. Above- and belowground productivity rates

242   were converted to N assimilation rates using the N content of triplicate seagrass biomass samples

243   that were collected simultaneously with the productivity samples at two plots using 15 cm

244   diameter, 15 cm depth cores. Biomass samples were sorted into live and dead above and

245   belowground biomass, dried to a constant weight at 60ºC, pulverized using a Biospec Products

246   MiniBeadBeater, and analyzed for N content on a Carlo Erba NA 2500 Elemental Analyzer.

247   Seagrass shoot density was also measured concurrently with productivity in 8-10 replicate 0.25

248   m^2 quadrats scattered haphazardly at each plot, and shoot-specific assimilation rates were scaled

249   to areal assimilation rates by shoot density.

250   **Results**

251   *N loading*

252   Table 2 shows the N loading values from Anderson et al. 2010 scaled to the lagoon and

253   watershed areas of South Bay in order to calculate the total N load to the South Bay meadow. On

254   an areal basis, the lagoon received a nitrogen load of 1.23 g N m^{-2} y^{-1} (95% confidence interval

255   (CI) 0.39 g N m^{-2} y^{-1}). Atmospheric deposition was the dominant source of nitrogen, accounting

256   for 77% of the external N inputs. Since these external inputs were not affected by the presence of

257   seagrass in the lagoon, the external N load was equivalent for both the seagrass and bare

258   sediment.

259   *N fixation and denitrification*

260   Daily rates of both N fixation and denitrification, scaled from the hourly rates reported

261   previously by Aoki and McGlathery (2018; 2019), peaked during summer (Figure 2). Total N

262   fixation rates, combining sediment and epiphyte rates, were greater than denitrification during

263 summer and similar during other seasons. Bare and seagrass sediments had comparable N

264 fixation rates, likely due to the similar organic matter content in the seagrass sediments and in

265 the experimentally cleared bare plots within the meadow (Aoki and McGlathery 2019). The

266 annual N fixation rate was 0.93 g N m^{-2} y^{-1} (95% CI 0.17 g N m^{-2} y^{-1}) in the seagrass meadow

267 and 0.74 g N m^{-2} y^{-1} (95% CI 0.16 g N m^{-2} y^{-1}) in the bare sediment. Daily rates of summer

268 denitrification were on average 4x greater in seagrass sediments than in bare sediments (Figure

269 2; note that denitrification rates were measured in both experimentally cleared plots within the

270 meadow and in bare sediments outside the meadow, see Aoki and McGlathery 2018 for details).

271 The annual denitrification rate was 0.62 g N m^{-2} y^{-1} (95% CI 0.21 g N m^{-2} y^{-1}) in seagrass

272 sediments and 0.16 g N m^{-2} y^{-1} (95% CI 0.05 g N m^{-2} y^{-1}) in bare sediments.

273 ***N burial***

274 As the seagrass meadow has matured over time, burial rates have increased due to

275 increased sedimentation rates and increased sediment N content (Figure 3). Burial rates within

276 each age bracket ranged from 0.17 g N m^{-2} y^{-1} (95% CI 0.14 g N m^{-2} y^{-1}) in bare sediments to

277 3.52 g N m^{-2} y^{-1} (95% CI 0.69 g N m^{-2} y^{-1}) in the oldest (>9 years old) meadow sediments (Table

278 3). Compared across age brackets, burial rates varied significantly (ANOVA, $F_{3,21}$=5.22,

279 p<0.05), and post-hoc contrasts showed that the >9 year old sediments had significantly higher

280 rates than both the bare and recently colonized (1-5 year old) sediments (Tukey test, p<0.05).

281 The total area of the seagrass meadow has also expanded steadily through 2015 (Figure 4), and

282 the oldest areas of the meadow accounted for only a small proportion of the total meadow area in

283 2015 (Table 3). The average burial rate over the total 2015 meadow area, weighted based on the

284 proportion of the meadow area in each age bracket, was 1.95 g N m^{-2} y^{-1}.

285 ***N retention***

286   Shoot-specific aboveground seagrass productivity peaked in June (Figure 5), leading to

287   peak N assimilation rates in summer (Figure 6). Shoot counts were highest in June 2017;

288   however, N content of aboveground tissue was highest in October 2016. Belowground tissue N

289   content and belowground biomass growth rates showed little variation across seasons. Assuming

290   a winter assimilation rate of half the fall assimilation rate (based on winter shoot densities

291   measured in previous years, data not shown), the annual assimilation rate was 2.62 g N m^{-2} y^{-1}.

292   **Discussion**

293   ***Burial dominates N removal***

294   Net N removal, the combination of burial and denitrification, was more than 12x greater

295   in the mature meadow sediments compared to bare sediments (4.14 compared to 0.33 g N m^{-2} y^{-}

296   ^{1}), demonstrating that the seagrass restoration successfully reinstated the N filter function. Burial

297   was the major component of N removal, accounting for 85% of the total. Recent work using

298   staple isotopes to characterize the sediment organic carbon (SOC) in the South Bay seagrass

299   meadow showed that approximately 50% of the SOC derived from benthic microalgae, 40%

300   from seagrass, and 10% from marsh grass (*Spartina alterniflora)* (Oreska et al. 2017b). A similar

301   breakdown of the sediment organic nitrogen pool would suggest that both the seagrass and

302   benthic microalgae were important sources of nitrogen buried in the seagrass sediments. Burial is

303   therefore one important link between the high productivity of the seagrass and the filter function.

304   The meadow assimilates large amounts of dissolved nitrogen into biomass during the growing

305   season and a portion of that biomass remains buried in the sediments, reducing the N available

306   for immediate recycling. Productivity is also linked to burial by the physical structure of above

307   and belowground seagrass biomass that directly enhances burial through wave attenuation and

308   sediment stabilization.

309   Our analysis showed that N burial increased with time since restoration (Table 3). The

310   maximum burial rate measured at the mature meadow sites (>9 years old) was 3.52 g N m^{-2} y^{-1},

311   which falls within the range of previously reported rates in *Zostera* meadows (2.7-3.9 g N m^{-2} y^{-}

312   ^{1}, Eyre et al. 2016, Table 1). Thus, within 10 years after seeding, the restoration successfully

313   reestablished N removal via burial to rates comparable to natural meadows. The meadow-wide

314   burial rate in 2015, calculated as a weighted average of burial rates in the different age classes

315   (time since restoration) of the meadow, was lower, 1.95 g N m^{-2} y^{-1}. If we extrapolate this current

316   meadow-wide burial rate to the full area of the meadow in 2015, 6.9 km^2, we would expect the

317   meadow to remove 13.6 t N y^{-1} through burial, while an equivalent area of bare sediment would

318   bury an order of magnitude less (1.2 t N y^{-1}). As the restored meadow continues to mature, we

319   expect that the burial rate will increase in younger areas of the meadow, leading to a meadow-

320   wide burial rate on par with natural systems. This finding mirrors earlier work demonstrating that

321   carbon burial in the restored meadow sediments was comparable to natural systems within ten

322   years of restoration (Greiner et al. 2013; Oreska et al. 2017a).

323   Denitrification was a minor component of the N filter in the seagrass sediments (15% of

324   net N removal), but denitrification rates in seagrass sediments were enhanced by approximately

325   4x compared to bare sediments. Many studies of N removal in seagrass meadows to date have

326   focused on enhanced denitrification; however, our results show that burial can be the more

327   important factor. The hourly rates used in this study agree well with numerous measurements of

328   denitrification in seagrass sediments, including both isotope pairing (e.g. Welsh et al. 2000,

329   Russell et al. 2016) and N$_2$:Ar studies (Zarnoch et al. 2017). However, the extrapolated annual

330   rate of denitrification (0.62 g N m^{-2} y^{-1}) is low compared to two recent studies using dark N$_2$:Ar

331   rates (7-19 g N m^{-2} y^{-1}; Smyth et al. 2013; Eyre et al. 2016). While the denitrification rates in this

332   system were likely limited by the very low nitrate availability (undetectable in surface waters

333   throughout the year) and relatively low sediment organic matter content (2.6%), it is not

334   immediately clear why the rates differ so dramatically between locations. Factors such as

335   residence time and benthic microalgae presence also influence denitrification rates (Cornwell et

336   al. 1999; Welsh et al. 2000), and methodological differences likely contribute to the wide range

337   of denitrification rates reported in the literature (see Eyre et al. (2013) and Aoki and McGlathery

338   (2017) for more discussion of how methodology affects denitrification measurements). Further

339   investigation of denitrification in seagrass meadows is needed to clarify the range of rates in

340   natural systems under different conditions and to establish the importance of denitrification

341   relative to N burial.

342   ***Significant N retention in seagrass biomass***

343   Over an annual time scale, seagrass N assimilation was a major flux, comparable to 75%

344   of the burial rate in the most mature areas (Table 4). In fact, this N assimilation rate likely

345   underestimates total N demand by the seagrass, since the leaf-marking technique used in this

346   study does not include leaf maturation (thickening and widening of the leaf) above the needle

347   scar in the growth rate (Park et al. 2010). The large N assimilation rate indicates how seagrass

348   biomass mediates N cycling in the lagoon. By retaining nitrogen in recalcitrant seagrass tissue

349   during the growing season, the meadow limits rapid recycling of nutrients that can fuel

350   phytoplankton and macroalgae blooms (Banta et al. 2004). Temporary N retention in seagrass

351   biomass thus provides an additional filter mechanism during the growing season that helps to

352   maintain water quality, creating an environment conducive to seagrass growth.

353   Although seagrass biomass is more persistent than algae, a portion of the N assimilated

354   into seagrass tissue will eventually be recycled. N recycling includes both remineralization of

355   seagrass-derived N in meadow sediments and internal recycling of N within seagrass shoots,

356   sometimes known as N reclamation. We did not measure remineralization or reclamation, but

357   previous studies indicate that each of these processes may meet as much as 50% of the seagrass

358   N demand (Romero et al. 2006 and references therein), and previous work has demonstrated that

359   remineralization is important to meet benthic microalgal demand in this system (Anderson et al.

360   2003). Generally, tightly linked recycling processes allow seagrass to achieve extremely high

361   rates of productivity in nutrient-poor waters, as was likely the case in our study system. The high

362   N assimilation rate measured here underscores the duality of seagrass meadows. As productivity

363   hotspots, they drive nutrient cycling, including the release of nutrients through remineralization.

364   At the same time, the meadows filter nitrogen through burial, stimulation of denitrification, and

365   retention in biomass. By creating a positive feedback for seagrass growth, the coastal filter

366   function supports the many other ecosystem services provided by seagrass meadows, such as

367   habitat provisioning and carbon sequestration, in addition to limiting water column nitrogen.

368   The fate of nitrogen assimilated into seagrass biomass is largely unknown. While a

369   portion of the nitrogen that accumulates in seagrass biomass is buried in seagrass sediments, an

370   additional portion is exported from the ecosystem. Leaves and shoots are lost throughout the

371   growing season, and, in this system, accumulations of seagrass wrack have been observed on the

372   ocean side of the barrier islands. Seagrass biomass may contribute to sediment organic matter in

373   the neighboring marshes, and a recent study suggests that seagrass wrack may be buried on the

374   continental shelf (Duarte and Krause-Jensen 2017). This physical transport of biomass nitrogen

375   out of the seagrass meadow, coupled with the low rates of external N inputs from the watershed,

376   underscores the importance of internal N loading from enhanced N fixation and remineralization.

377   ***Low N inputs from loading and fixation***

378   The N inputs from watershed loading were low compared to the N removal and retention

379   fluxes (Table 4). Previous measurements have shown that the Virginia coastal bays experience

380   some of the lowest rates of watershed N loading compared to coastal lagoons worldwide

381   (McGlathery et al. 2007) so the low rates shown here were expected. N fixation, an internal

382   source of N within the meadow, was also low compared to total N removal and retention fluxes,

383   although fixation did outweigh denitrification, both during peak summer rates and in the

384   integrated annual rate. The low rates of N loading likely contributed to the dominance of N

385   fixation over denitrification; NO_3^- concentrations in surface waters in South Bay were often

386   undetectable (unpublished data), limiting the supply of nitrate to support denitrification. The

387   underlying hourly rates of N fixation used in this analysis were similar to rates measured in other

388   *Zostera* meadows (Welsh et al. 1996; McGlathery et al. 1998; Russell et al. 2016). Few authors

389   have scaled hourly rates to annual, areal rates, although Russell et al. (2016) recently found that

390   N fixation dominated denitrification both hourly and over an annual time-scale in intertidal *Z.*

391   *muelleri* flats. Overall, the low rates of N fixation were in line with expectations from the

392   literature.

393   Given the high rates of burial and assimilation and low rates of N loading and fixation

394   measured in this study, it is clear that additional sources provided nitrogen to the meadow. As

395   mentioned above, internal recycling (i.e. remineralization and N reclamation) were likely

396   important sources of N to the seagrass meadow. However, there is also evidence to suggest that

397   the N fixation rates used in this study were underestimated. Acetylene reduction, the traditional

398   method used here to measure N fixation in sediments, is an indirect method with several known

399   drawbacks (Welsh 2000; Fulweiler et al. 2015). Recent studies using direct measurement

400   techniques in estuarine sediments have found substantially higher N fixation rates compared to

401 published acetylene reduction values in both unvegetated sediments (Gardner et al. 2006;

402 Fulweiler et al. 2007; Newell et al. 2016), and in the South Bay meadow (Aoki and McGlathery

403 2019). Additional direct measurements of N fixation are needed to explore these patterns and to

404 fully account for internal N inputs that support the high rates of N assimilation in the meadow.

405 **Motivation for restoration and conservation**

406 Seagrass meadows around the world are threatened by human activity. Both restoration

407 of lost meadows and conservation of existing meadows are needed to preserve these important

408 ecosystems and the many functions they perform (Unsworth et al. 2018). This study shows that

409 seagrass restoration can recover the N filter function within ten years of seeding, reestablishing

410 positive feedbacks that support continued seagrass growth. While the exact rates measured here

411 may not translate to other restored systems, the relative importance of different N filter processes

412 is instructive.

413 Overall, our results indicate that burial was by far the dominant process removing N from

414 the system, accounting for 85% of total removal. Seagrass productivity was the second most

415 important component of the N filter function, temporarily retaining N during the growing season

416 and improving water quality. These two processes are linked through the physical structure of

417 the canopy and the contribution of seagrass tissue to the sediment N pool. However, burial rates

418 in the most mature meadow sites, i.e. sites comparable to an established natural meadow,

419 exceeded N assimilation into seagrass biomass by 1.3x. Rates of both burial and assimilation

420 measured here were likely low compared to other eelgrass systems. The Virginia coastal bays are

421 near the southern end of the geographic range of *Z. marina* (Moore and Short 2006), and eelgrass

422 meadows in more northern climates have shown even greater productivity rates (Pedersen and

423 Borum 1993; Risgaard-Petersen et al. 1998). Leaf N content also indicated that the eelgrass in

424   South Bay were N limited at least part of the year (Figure 5B, values below 1.8% indicate

425   nitrogen limitation, Duarte 1990). In systems with greater N loading that relieves this limitation,

426   seagrass assimilation rates would be somewhat greater. Burial rates could also be much greater

427   than the rate reported here in more eutrophied systems with greater seston concentrations; under

428   eutrophied conditions, burial would likely exceed assimilation by even more than in this

429   oligotrophic system. To date, burial rates in *Zostera* meadows have been measured only under

430   oligotrophic conditions (Eyre et al. 2016 and this study), and more measurements of N burial in

431   temperate seagrass meadows are needed to quantify the range of burial rates across different

432   environmental conditions.

433   The importance of seagrass ecosystems as sites for N burial can motivate both restoration

434   and conservation. In the restored meadow studied here, the return of mature seagrass increased

435   the capacity of the system to remove N by 20x compared to unvegetated sediments. This large N

436   removal capacity in turn could potentially limit negative effects from external N loading.

437   Conversely, in areas where seagrass meadows are lost, the system would have a diminished

438   capacity to retain N, leading to an increase in available N that is independent of increases in

439   external N loading rates. The results of this study therefore emphasize the importance of both

440   restoration and conservation of seagrass meadows in order to maintain the coastal N filter

441   function. Better quantification of N removal rates in both natural and restored meadows is

442   needed to add to the results presented here and to further our understanding of how seagrass

443   influence N removal rates.

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Acknowledgements

627 **Figure Legends**

628 **Figure 1.** Map of the South Bay meadow showing the original seeded plots and meadow

629 expansion over time.

630 **Figure 2**. Daily rates of denitrification (negative values, filled bars) and N fixation (positive

631 values, patterned bars) scaled from hourly measurements reported previously by Aoki and

632 McGlathery (2018 and 2019). Measurements in seagrass sediments are light grey; measurements

633 in bare sediments are dark grey. Error bars are SE, n=18-22 for summer values, n=6-9 for other

634 seasons.

635 **Figure 3.** Accretion rates (from Greiner et al. 2013) and sediment N content increased with

636 meadow age (time since restoration). Error bars are SE, n=3 (bare), 8 (1-5 years), 4 (5-9 years),

637 10 (>9 years).

638 **Figure 4** The restored meadow expanded rapidly through 2015, leading to spatial variability in

639 the age (time since restoration) of the meadow.

640 **Figure 5.** Shoot-specific productivity (A), seagrass tissue N content (B), shoot-specific

641 elongation (C), and seagrass shoot density (D) were measured seasonally from June 2016-August

642 2017. Error bars are standard error, n=3 for Oct, April, August, n=6 for June measurements.

643 **Figure 6.** Areal N assimilation rates were calculated from the shoot-specific productivity, N

644 content, and shoot density (shown in Figure 5). Error bars are standard error, n=6 in summer,
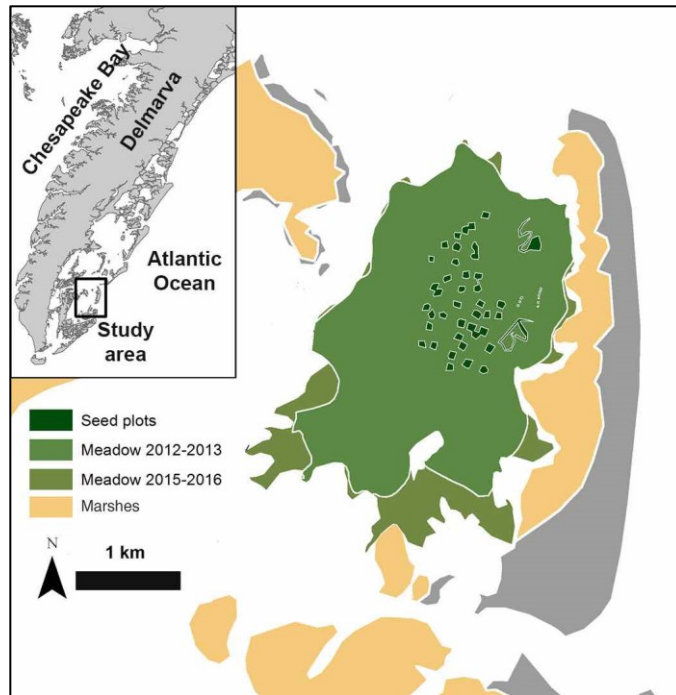
645 n=3 in fall and spring.

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Figure 1. Map of the South Bay meadow showing the original seeded plots and meadow
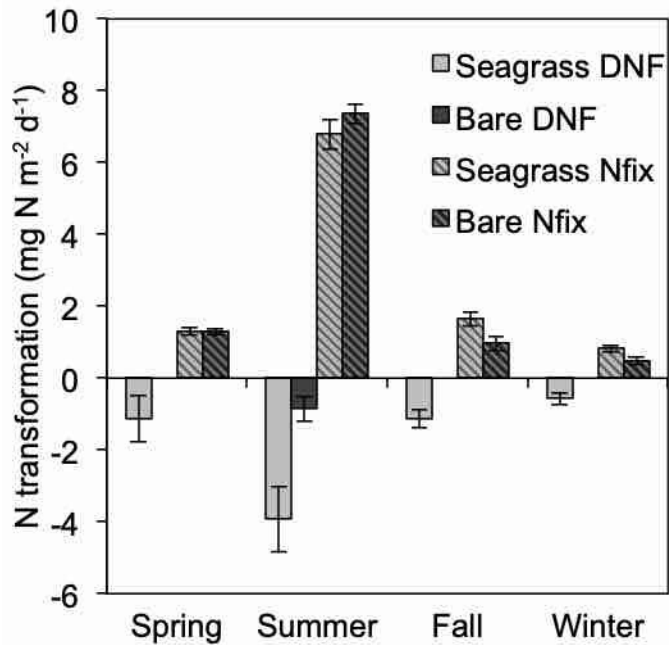
649   expansion over time.

650

Figure 2. Daily rates of denitrification (negative values, filled bars) and N fixation (positive values, patterned bars) scaled from hourly measurements reported previously by Aoki and McGlathery (2018 and 2019). Measurements in seagrass sediments are light grey; measurements in bare sediments are dark grey. Error bars are SE, n=18-22 for summer values, n=6-9 for other seasons.
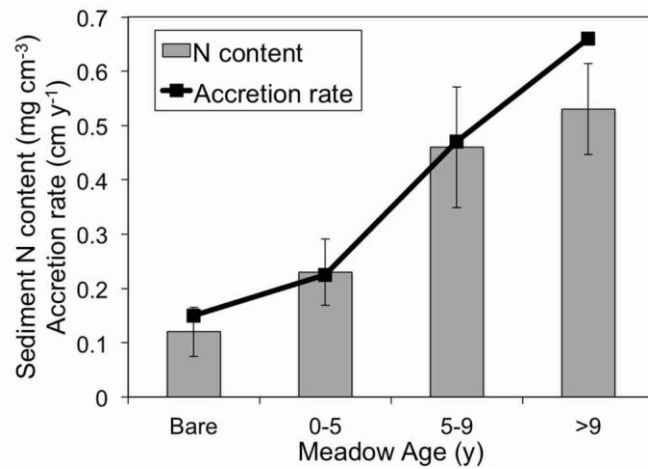
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Figure 3. Accretion rates (from Greiner et al. 2013) and sediment N content increased with

meadow age (time since restoration). Error bars are SE, n=3 (bare), 8 (1-5 years), 4 (5-9
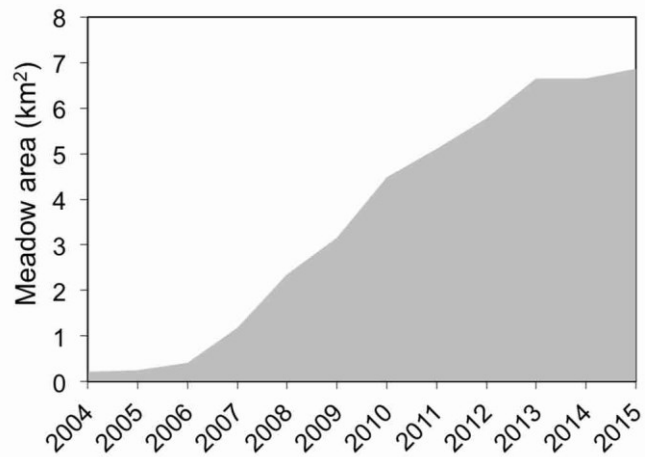
years), 10 (>9 years).

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Figure 4 The restored meadow expanded rapidly through 2015, leading to spatial variability in

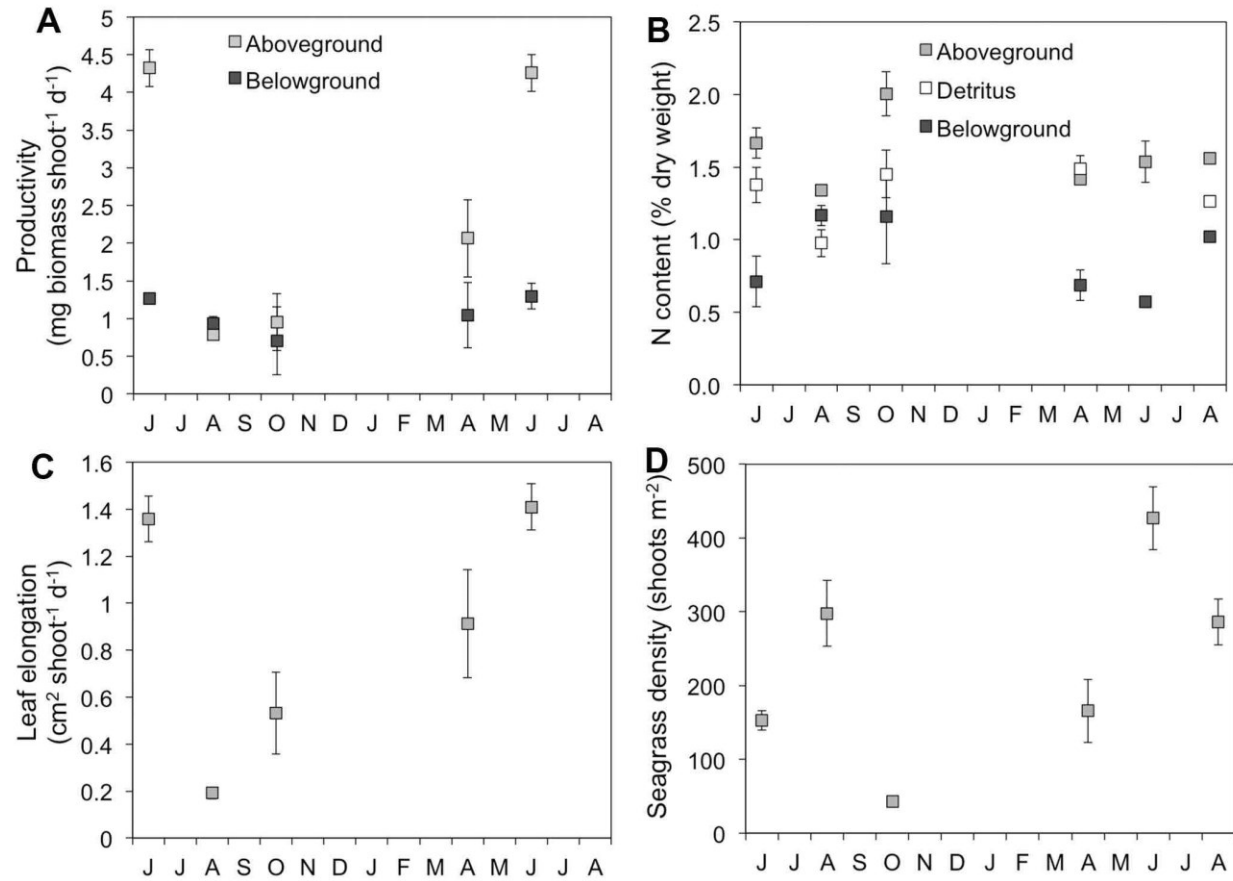663 the age (time since restoration) of the meadow.

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Figure 5. Shoot-specific productivity (A), seagrass tissue N content (B), shoot-specific

elongation (C), and seagrass shoot density (D) were measured seasonally from June 2016-

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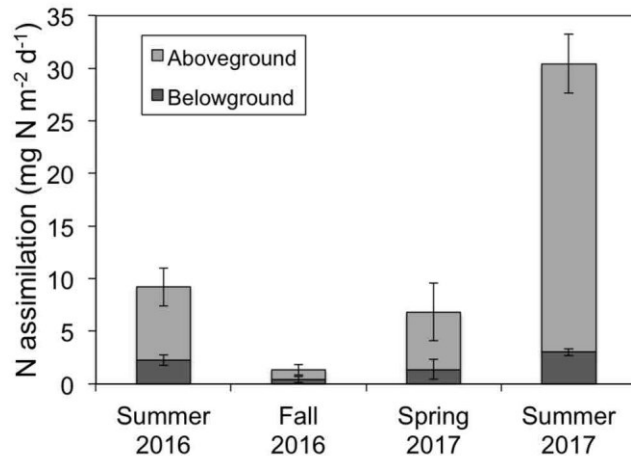
measurements.

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671

672 **Figure 6.** Areal N assimilation rates were calculated from the shoot-specific productivity, N

673 content, and shoot density (shown in Figure 5). Error bars are standard error, n=6 in summer,

674 n=3 in fall and spring.

675

676 **Tables**

677 **Table 1.** Rates of N burial in seagrass meadow sediments have so far been reported mainly for

678 *Posidonia* meadows and mainly for oligotrophic ecosystems

Location	Species	N burial $(g \, N \, m^{-2} \, y^{-1})$	Source
SE Australia[†]	*Zostera* sp.	2.7-3.9	Eyre et al. 2016
	Halophila sp.	1.3-8	
	Ruppia sp.	1.8-8.2	
	Posidonia sp.	5.8	
NW Mediterranean	*Posidonia oceanica*	13.4	Gacia et al. 2002
NW Mediterranean	*Posidonia oceanica**	0.5-1.1	Mateo and Romero 1997
NW Mediterranean	*Cymodocea nodosa*	3.57	Pedersen et al. 1997
Central Mediterranean	*Posidonia oceanica**	0.2-2.3	Romero et al. 1994
Virginia coastal bays, USA[†]	*Zostera marina*	3.52	This study

679 *Burial rate of seagrass detritus only, excluding particulate N in sediment
680 †Measurements made in oligotrophic systems

681   **Table 2.** N Loading values were adopted from Anderson et al. 2010, and loads were calculated

682   based on catchment and lagoon areas; loading rates are shown as mean (95% CI min-max).

N Source	N loading (kg N ha^{-1} y^{-1})	Area (ha)	N load (kg N y^{-1})
Catchment		6037	47000
Base Flow	4.59 (0.86-8.76)		
Surface water runoff	1.84 (0.34-3.50)		
Groundwater discharge	1.51 (0.28-2.89)		
Lagoon		16946	161000
Atmospheric deposition	9.49 (7.97-11.01)		
Total			208000

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685   **Table 3.** Burial rates were calculated from the accretion rate and N content for different ages of

686   restored meadow; values are mean (SE), n=3 (bare), 8 (1-5 years), 4 (5-9 years), 10 (>9 years).

	Bare sediment	1-5 years old	5-9 years old	>9 years old
Burial (g m^{-2} y^{-1})	0.17 (0.07)	0.53 (0.14)	2.16 (0.52)	3.52 (0.55)
Proportion of meadow area in 2015	--	26%	57%	17%

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689　**Table 4.** Comparison of annual N inputs, N retention, and N removal in the meadow and bare

690　sediments[*]

	N flux	Mean rate	95% confidence	Source
		$(g\ N\ m^{-2}\ y^{-1})$	limit	
Seagrass				
	Loading	1.23	0.39	Adapted from Anderson et al. 2010
	Fixation	0.93	0.17	Aoki & McGlathery 2019
	Denitrification	-0.62	0.21	Aoki & McGlathery 2018
	Burial	-3.52	0.69	This study
	Assimilation	-2.62	0.78	This study
Bare				
	Loading	1.23	0.39	Adapted from Anderson et al. 2010
	Fixation	0.74	0.16	Aoki & McGlathery 2019
	Denitrification	-0.16	0.05	Aoki & McGlathery 2018
	Burial	-0.17	0.14	This study

691　[*]Positive rates indicate N inputs, negative rates indicate N removal and retention.
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