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Effects of inorganic nutrient P and N application on *Azolla* biomass growth and nutrient uptake

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Abstract

Rice farmers in Mwea Irrigation Scheme routinely apply P and N fertilizers which affect water nutrient levels. A study was conducted to establish the effects of nutrient N and P application on *Azolla* biomass accumulation. The study was conducted in a batch culture experiment, using 5g of fresh *Azolla* biomass samples from each of the six major paddy schemes namely: Mwea, Ahero, West Kano, Bunyala, TARDA, and Taveta. Treatments consisted of 0 and 3mg P l^{-1} and 0 and 200mg N l^{-1}, laid out in a randomized complete block design replicated three times. *Azolla* samples were grown in batch culture plastic pots of 8.4 x $10^{-3}m^3$ for 10 days using canal water, which was replenished every three days. Fresh *Azolla* biomass weight was recorded at 0, 5 and 10 days after inoculation. Data was subjected to analysis of variance using SAS statistical package version 9.1 and means separated using the least significant difference test ($p \leq 0.05$). The pH levels in irrigation water averaged 7.2 while N, P and K levels were 20.2, 11.6 and 15ppm respectively. Tissue N and K for *Azolla* accession averaged 4.2% and 1.6% respectively. Biomass accumulation and doubling time of *Azolla* were significantly affected by exogenous P and N nutrient application. Doubling time ranged from 5.5 days to 6.7. Application N and P significantly reduced *Azolla* biomass accumulation and increased biomass doubling time.

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Introduction

Azolla is a pteridophyte which forms a symbiotic association with a cyanobacterium-*Anabaena azollae* (Bocchi *et al*., 2010) and fixes nitrogen at a rate higher than legumes (Wagner, 1997). A study on *Azolla* has reported that *Azolla* contains 4.5% N, 0.4% P and 1.5-3% K (Watanabe, 1989). In Mwea Irrigation Scheme, the existing species was found to contain 3.9% N, 0.44% P, and 1.08% K (unpublished). *Azolla* is capable of providing 40kg N ha^{-1} to the rice crop due to its nitrogen content which is released upon decomposition (Kannaiyan *et al*., 1982) reported that. This can reduce the cost of rice crop inorganic fertilizer, which constitutes 20% of rice production cost in Mwea Irrigation Scheme (Rice MAPP, 2012).

In Mwea Irrigation Scheme, *Azolla* coverage is estimated at 30-50% during peak times and this is majorly dependent upon water availability (unpublished). Nutrient status and other environmental factors are major factors affecting *Azolla* biomass growth (Wagner, 1997). Extensive and intensive inorganic fertilizer use forms the primary source of the water nutrient status and eutrophication in water bodies (FAO/ECE, 1991). Depending on the levels, this can cause atmospheric, aquatic and ground water system pollution (Choudhury *et al.,* 2005). Farmers in Mwea irrigation scheme apply estimated P and N fertilizer amounts of 58kg of P_2O_5 and 56kg of N per hectare respectively, based on recommendations by Wanjogu *et al.* (1997). These fertilizers applied contribute to the water nutrient status being conducive for *Azolla* growth.

The nitrogen fixing ability of *Azolla* makes it able to grow in nitrogen deficient waters (Watanabe, 1979; Hussner, 2010). However, its growth is limited by the nutrient element P (Kitoh *et al.,* 1993). Kondo *et al.* (1989) reported a maximum growth rate for *Azolla* under application of 3.1ppm P with a threshold limit of 0.5-0.6% P. The level of phosphorus in water bodies is varied and can be high due to fertilizer use and runoff. This can lead to *Azolla* blooms in paddies or flood waters. Depending on the water nutrient status, *Azolla* multiplies fast; doubling its biomass in less than 10 days and readily colonizes new areas (Campbell, 2011). Hussner (2010) reported a doubling rate of 3-10 days, while Kitoh (1993) reported a doubling rate of 2-3 days under laboratory conditions.

The nutrient P is important for *Azolla* growth and reproduction (Sadeghi, 2012). Its deficiency has been shown to hinder the acetylene reduction activity (Tung *et al*., 1989). However, excess levels of nutrient P has been reported to have a negative effect on *Azolla* growth (Pitt *et al*., 2014). According to Rains *et al.* (1979), a P level of 0.34ppm is the lower threshold limit below which there is deficiency. Subudhi *et al.,* (1981) reported that external P level of 5ppm is the higher threshold limit beyond which *Azolla* tissue N content is affected negatively. The nutrient N is important for *Azolla* growth but because of its N fixing ability, it is capable of growing in N free media (Hussner, 2010). External N has been shown to inhibit the activity of acetylene reductase activity (Yatazawa *et al*., 1980). Kitoh, 1991 showed that external ammonia N negatively affects *Azolla* growth and N fixation activity. The objective of this study was to determine the effects of P and N on *Azolla* biomass growth and tissue N and P uptake, in Mwea Irrigation Scheme.

Materials and methods

Experimental site

The study was conducted in Mwea Irrigation Scheme, at Mwea Irrigation and Agricultural Development Centre (MIAD), in Kirinyaga County at an altitude of 1159 metres above the sea level. The climate is tropical with equatorial and medium high altitude characteristics and it lies within Agro-ecological zones LM3 and LM 4 (Marginal cotton zones). Mwea receives an average of 930mm of rainfall, out of which 510mm is received during the long rainy season and 290mm in the short season, with 66% reliability. The mean temperature is 22^{o}C with a minimum of 17^{o}C and maximum of 28^{o}C. The soils are predominantly vertisols (LB 8) imperfectly drained, deep dark grey to black, cracking with calcareous deep sub soil. During the experimental period, the average temperatures were 21^{o}C and 22^{o}C for the first and second season respectively. The relative humidity was 80% in the first season and 77% in the second season (Fig. 1).

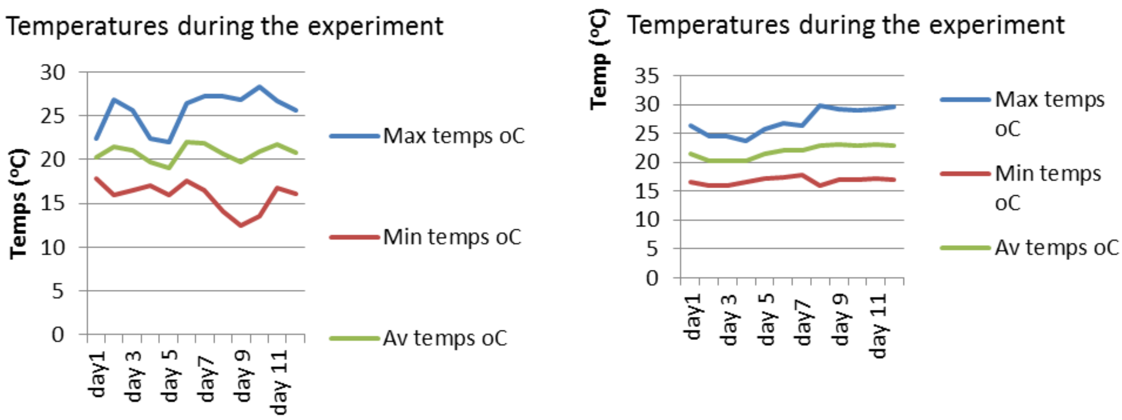

Fig. 1. Weather during the experimental period.

Experimental design and treatments

The treatments consisted of seven *Azolla* samples collected from six major irrigation schemes (Mwea, Ahero, West Kano, Bunyala, Taveta and TARDA), four fertilizer levels (0 and 3mg P l^{-1}, 0 and 200mg N l^{-1}). The experiment was laid out in a randomized complete block design, replicated three times. About 5 g of fresh *Azolla* biomass sample each from the five major irrigation schemes in Kenya (Ahero, West Kano, Bunyala, Taveta and TARDA), were inoculated and grown in plastic pots of 8.4 x $10^{-3}m^3$ (30.5cm diameter x 11.5cm height) for 10 days using Mwea irrigation canal water. Canal water was collected from irrigation drainage canals within Mwea Irrigation Scheme rice fields and was replenished every 3 days to avoid depletion of nutrients.

Data collection

Fresh *Azolla* biomass weight was recorded at inoculation and 10 days later after washing with running water. Doubling time was calculated based on Kitoh and Shiomi (1991) equation: DT = tlog 2/ GV, Where t= duration (day) of culture, GV= wet weight of harvested plants/ wet weight of initial plants. Temperature and relative humidity was recorded during the experiment. About 100g of *Azolla* biomass and 500ml of canal water were collected from each of the irrigation canals of the six major rice schemes. *Azolla* biomass collected was analysed for tissue N, P and K before and upon termination of the experiment. Irrigation water was analysed for N, P, K, pH and Ec.

Azolla tissue N content was determined using Kjedahl's method (Kjedahl, 1883), P content by calorimetric method (Barton, 1948) and K content using flame photometry method (Schollenberger *et al.*, 1945). From the irrigation water samples, N content was analysed using the procedure by Alpha (Alpha, 1965), P content by Orthophosphate method (Jackson, 1958), K content by atomic absorption method (Fishman, 1965) and pH by glass electrode method (Jackson, 1958).

Data analysis

Data were subjected to analysis of variance using SAS statistical package (version 9.1) and means separated using the least significant difference test at $p \leq 0.05$. Linear regression analysis was done to establish the relationship between *Azolla* tissue N and irrigation water N.

Results

Irrigation water nutrient quality

The levels of N, P and K in the irrigation water ranged from 9.6-30.8ppm, 0.7-1.85ppm and 4-26ppm respectively (Table 1). Ahero irrigation water had significantly the highest levels of N and P, followed by West Kano. TARDA 2 water had significantly the highest level of P. The pH levels ranged from 6.9 -7.5. Ahero irrigation water had significantly the highest pH (7.1) while Bunyala was least (6.6). Taveta 2 irrigation water had significantly the highest chloride content (5.5me/l)) followed by Taveta 1 (4.7me/l) and least was Bunyala (2.7me/l).

Table 1. Irrigation water qualities for Mwea, Ahero, West Kano, Bunyala, Taveta and TARDA rice schemes in Kenya during the year 2017.

Accession	N (ppm)	P (ppm)	K (ppm)	pH	Na (ppm)	Cl (Me/l)	Ec 25° C (ds/m)
Mwea	10.9	0.8	4.5	6.8	84	3.7	0.8
Ahero	28.8	1.8	10.0	7.1	77.0	4.5	0.8
West Kano	22.5	1.2	14	6.8	92.0	3.4	0.8
Bunyala	14.3	1.2	4.3	6.6	66.0	2.7	0.6
Taveta 1	14.4	0.7	6.5	6.8	71.0	4.7	0.6
Taveta 2	10.5	0.7	7.3	6.8	82.0	5.5	0.6
TARDA 2	10.9	1.1	24.5	6.8	81.5	4.5	0.5
Mean	16.0	1.1	10.2	6.8	79.1	4.1	0.7
P-value	0.001	0.001	0.001	0.003	0.001	0.0002	0.0001
LSD(0.05)	3.7	0.13	3.0	0.13	6.8	0.6	0.06
CV (%)	9.3	4.9	11.9	0.8	3.5	5.5	3.5

Nitrogen, Phosphorus and potasium content of Azolla tissue on dry weight basis

The percentage nitrogen content of the different accessions is shown in Table 2. The nitrogen content on dry weight basis averaged 3.9%. Ahero accession had significantly the highest N (5.06%) followed by West Kano (4.8%) and Mwea (4.0%) accessions. Bunyala, Taveta 1, Taveta 2 and TARDA accessions had no significant differences in N levels. Mwea accession also had significantly the highest tissue P levels followed by TARDA 2 and Bunyala accessions. Taveta 2 accession had no significant differences in P levels with Ahero, accessions, while West Kano had significantly the least levels. Ahero accession had significantly the highest K levels, while Mwea was least. Taveta 2 and TARDA 2 had no significant differences in tissue% K.

Table 2. Nutrient content of *Azolla* accessions in Kenya.

Accession	N (%)	P(ppm)	K (%)
Mwea	4.0	4495.5	1.1
Ahero	5.1	2143.0	2.2
West Kano	4.8	1754.0	1.6
Bunyala	3.4	2312.5	1.5
Taveta 1	3.2	1990.0	1.3
Taveta 2	3.4	2187.5	2.0
TARDA 2	3.4	3913.5	1.9
Mean	3.9	2685.0	1.6
P-value	<0.001	<0.001	<0.001
LSD (0.05)	0.2	137.6	0.2
CV (%)	1.9	2.1	3.8

N(%)= percentage nitrogen on dry weight basis.

Regression relationship between Azolla tissue and Irrigation water N and P nutrient

Linear regression relationship showed a positive and significant relationship between *Azolla* tissue N and irrigation water N ((r=0.82) and a negative relationship between tissue P and irrigation water P (Fig 1 and Fig 2).

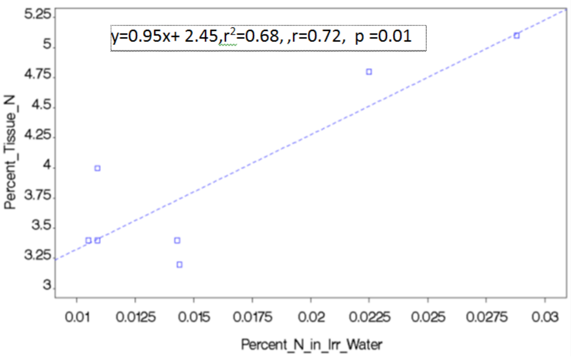

Fig. 2. Linear regression between *Azolla* tissue N and irrigation water N.

Effects of accessions and N on Azolla biomass growth rate

During the first season, the effect of N and the interaction between accession and N had significant effects on *Azolla* biomass growth (Table 3). Whereas application of 200mg l^{-1} N significantly reduced biomass accumulation for Ahero, West Kano, Bunyala, and Taveta 1 accessions, it significantly increased this parameter for Mwea, Taveta 2 and TARDA 2 accessions. At both N levels, Mwea accession had significantly the highest *Azolla* biomass.

Table 3. Effect of interaction between accessions and N on *Azolla* biomass accumulation.

	Season 1 Biomass accumulation (g)			Season 2 Biomass accumulation (g)		
	0mg N l^{-1}	200mg N l^{-1}	Mean	0mg N l^{-1}	200mg N l^{-1}	Mean
Mwea	25.7	29.4	27.6	20.4	13.7	17.1
Ahero	21.8	20.0	20.9	20.6	12.4	16.5
Wkano	21.1	15.0	18.1	15.2	8.9	12.1
Bunyala	25.1	17.5	21.3	17.5	12.4	15.0
Taveta1	20.8	18.2	19.5	17.8	11.4	14.6
Taveta 2	15.2	19.8	17.5	18.1	12.3	15.2
TARDA 2	13.7	18.2	16.0	21.3	12.9	17.1
Means	20.5	19.7		18.7	12.0	
P-value N	0.43			0.0001		
P-value accession	0.0001			0.007		
P-value accession x N	0.0007			0.8700		
LSD (0.05) N	NS			1.5		
LSD (0.05) accession	0.8			1.9		
LSD (0.05) accession x N	1.1			NS		
CV (%)	20.4			21.9		

During the second season, the interaction effect of accession and N was not significant but the main effects of N and accessions on biomass growth were significant. Application of 200mg l^{-1} N significantly reduced biomass accumulation for all accessions. West Kano accession had significantly the least biomass accumulation whereas TARDA 2 had significantly higher biomass than other treatments.

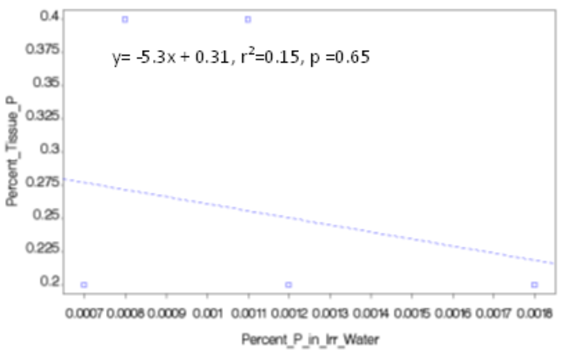

Fig. 3. Linear regression relationship between *Azolla* tissue P and irrigation water P.

Effects of accessions and N on Azolla biomass doubling rate

The accessions and the interaction between accessions and N had significant effect on biomass doubling rate during both seasons. However, the main effect of N was significant only during the second season (Table 4).

Table 4. Effect of interaction between accessions and N on *Azolla* biomass doubling time.

Accession	Season 1			Season 2		
	Biomass Dt (days)			Biomass Dt (days)		
	0mg N l^{-1}	200mg N l^{-1}	Mean	0mg N l^{-1}	200mg N l^{-1}	Mean
Mwea	4.3	4.1	4.2	4.5	6.6	5.6
Ahero	4.9	5.2	5.1	4.6	8.2	6.4
Wkano	5.0	6.7	5.9	5.9	13.8	9.9
Bunyala	4.4	6.4	5.4	5.2	7.4	6.3
Taveta1	4.9	5.2	5.1	5.0	8.3	6.7
Taveta 2	6.5	5.1	5.8	5.0	7.3	6.2
TARDA 2	7.8	5.7	6.8	4.6	7.8	6.2
Means	5.4	5.5		5.0	8.5	
P-value N	0.390			0.001		
P-value accession	0.0001			0.0004		
P-value accession x N	0.0001			0.0390		
LSD (0.05) N	NS			1.0		
LSD (0.05) accession	0.8			1.9		
LSD (0.05) accession x N	1.1			2.6		
CV (%)	18.7			33.7		

Dt= doubling time

During the first season, application of 200mg N l^{-1} significantly reduced doubling time for Taveta 2 and TARDA 2 accessions but significantly increased doubling time for West Kano and Bunyala accessions. At 0mg N l^{-1} TARDA accession had significantly higher doubling time than all other accessions. West Kano and Bunyala accessions had longer doubling time than other accessions at 200mg N l^{-1}. During the second season, application of 200mg N l^{-1} significantly increased doubling time all except Mwea accessions.

Effects of accessions and P on Azolla biomass growth and doubling rate

The effect of accessions on biomass growth was significant during both seasons while the effect of P on biomass growth was significant only during the second season. The interaction between P and accessions had no significant effect on biomass growth during both seasons (Table 1.5).

Table 5. Effects of interaction between accessions and P on *Azolla* biomass growth.

	Season 1			Season 2		
	Biomass accumulation (g)			Biomass accumulation (g)		
	0mg P l^{-1}	3mg P l^{-1}	Means	0mg P l^{-1}	3mg P l^{-1}	Means
Mwea	26.4	28.7	27.6	17.4	16.7	17.1
Ahero	20.7	21.0	20.9	19.4	13.5	16.5
Wkano	16.8	19.3	18.1	12.8	11.3	12.1
Bunyala	20.3	22.3	21.3	17.3	12.6	15.0
Taveta1	18.9	20.2	19.6	15.5	13.7	14.6
Taveta 2	18.7	16.3	17.5	16.1	14.2	15.2
TARDA 2	17.1	14.7	15.9	20.5	13.6	17.1
Means	19.8	20.4		17.0	13.7	
P-value P	0.59			0.0001		
P-value accession	0.0001			0.007		
P-value accession x P	0.63			0.19		
LSD (0.05) P	NS			1.45		
LSD (0.05) accession	3.5			2.7		
LSD (0.05) accession x P	NS			NS		
CV (%)	20.4			21.8		

In the first season, Mwea accession had significantly the highest biomass accumulation followed by Ahero accession. TARDA 2 had significantly lower biomass accumulation then Ahero and Bunyala accessions. In the second season, at 3.0mgkg^{-1} P treatment, Mwea accession had significantly the highest biomass accumulation but not significantly different from Taveta 2 accession. Treatment with 3mgkg^{-1} P significantly reduced biomass growth for Ahero, Bunyala, Taveta 1, Taveta 2 and TARDA 2 accessions. There was no significant effect on biomass growth for Mwea and West Kano accessions. Phosphorus application had no effect on biomass accumulation.

Effects of accessions and P on Azolla biomass doubling time

The effect of accessions on biomass doubling time during both seasons was significant while P effect on biomass was not significant.

The interaction effect of accessions and P was significant on doubling rate during the first season (Table 6). In the first season, application of P significantly reduced biomass doubling time for West Kano and Bunyala accessions while it significantly increased the parameter for TARDA 2 accession. Mwea, Ahero, Taveta 1 and Taveta 2 accessions were not significantly affected. TARDA 2 accession had significantly longer doubling time than most of the accessions. In the second season West Kano accession had significantly the longest doubling time.

Table 6. Effect of accession and P on biomass doubling time.

	Season 1			Season 2		
	0mg P l^{-1}	3mg P l^{-1}	Means	0mg P l^{-1}	3mg P l^{-1}	Means
Mwea	4.3	4.1	4.2	5.6	5.4	5.5
Ahero	5.2	4.9	5.1	5.0	7.8	6.4
Wkano	6.5	5.2	5.9	10.9	8.9	9.9
Bunyala	6.1	4.7	5.4	5.4	7.2	6.3
Taveta1	5.8	5.1	5.5	6.3	7.0	6.7
Taveta2	5.6	6.1	5.9	6.0	6.3	6.2
TARDA 2	5.8	7.6	6.7	4.8	7.5	6.7
Means	5.5	5.4		6.7	7.2	
P-value P	0.26			0.08		
P-value accession	0.0001			0.0004		
P-value accession x P	0.005			0.126		
LSD (0.05) P	NS			NS		
LSD (0.05) accession	0.8			1.85		
LSD (0.05) accession x P	1.2			NS		
CV (%)	17.7			33.6		

Effect of interaction between P and N on Azolla biomass growth.

During the first season, the effect of interaction between P and N on *Azolla* biomass growth and was significant (Table 7). At 0mg P l^{-1}, 200mg N l^{-1} application significantly reduced biomass growth while at 3mg P l^{-1}, N application significantly increased biomass growth. During the second season, the interaction effect between N and P was not significant but N and P application significantly affected biomass growth. Application of 200mg N l^{-1} significantly reduced *Azolla* biomass growth under both 0mg P l^{-1} and 3mg P l^{-1} treatments.

Effects of accession and N on Azolla tissue N

The effects of accessions, N and the interaction between accessions and N were significant for tissue N during both seasons (Table 8). During the first season, 200mg N l^{-1} significantly increased tissue N for all accessions. Ahero accession had significantly higher tissue N than Taveta 1 accession. Taveta 1 had significantly the least tissue N but not significantly different from Bunyala and TARDA 2. During the second season, treatment with 200mg l^{-1} N also significantly increased tissue N in all accessions except for TARDA 2 accession. TARDA 2 had significantly the least tissue N No significant differences were observed amongst the other accessions.

Table 7. Effect of interaction between P and N on *Azolla* biomass growth rate.

	Season 1			Season 2		
	0mg N l^{-1}	200mg N l^{-1}	Means	0mg N l^{-1}	200mg N l^{-1}	Means
0mg P l^{-1}	22.4	17.3	19.9	20.8	13.2	17.0
3mg P l^{-1}	18.6	22.2	20.4	16.6	10.8	13.7
Mean	20.5	19.75		18.7	12.0	
P-value P	0.59			0.0001		
P-value N	0.43			0.0001		
P-value P x N	0.0001			0.223		
LSD (0.05) P	NS			1.45		
LSD (0.05) N	NS			1.5		
LSD (0.05) P x N	2.5			NS		

Table 8. Effect of interaction between accession and N on *Azolla* tissue N.

Accession	Season 1			Season 2		
	Treatment			Treatment		
	0mg N l^{-1}	200mg N l^{-1}	Mean	0mgN l^{-1}	200mg N l^{-1}	Mean
Mwea	3.4	4.7	4.1	2.7	4.0	3.4
Ahero	3.3	5.1	4.2	2.7	4.0	3.4
Wkano	3.1	5.1	4.1	2.7	4.1	3.4
Bunyala	3.0	4.8	3.9	3.0	3.9	3.5
Taveta1	2.8	4.6	3.7	3.2	4.2	3.7
Taveta 2	3.5	4.6	4.05	3.1	4.1	3.6
TARDA 2	3.5	4.5	4.0	3.0	2.7	2.9
Means	4.0			3.4		
P-value N	0.0001			0.0001		
P-value accession	0.0054			0.0001		
P-value accession x N	0.0001			0.0049		
LSD (0.05) accession	0.19			0.18		
LSD (0.05) accession x N	0.26			0.25		
CV (%)	7.2			4,8		

Effects of accession and P on Azolla tissue P

The effects of P, accessions and the interaction between P and accessions on tissue P were significant on tissue P during both seasons (Table 9). During the first season, treatment with 3mg P l^{-1} significantly increased tissue P for all accessions. West Kano accession had significantly the highest tissue P accumulation while Mwea accession had significantly the lower tissue P accumulation than most accessions. In the second season, 3mg P l^{-1} significantly increased tissue P in all the accessions. TARDA 2 accession had significantly the highest tissue P while Mwea accession had significantly the least tissue P but not significantly different from that of West Kano and Taveta 2 accessions.

Table 9. Effect of interaction between accession and P on *Azolla* tissue P.

	Season 1			Season 2		
	P treatment			P treatment		
	0mg l⁻¹ P	3mg l⁻¹ P	Means	0mg l⁻¹ P	3mg l⁻¹ P	Means
Mwea	1421.5	3558.3	2489.9	1863.5	3660.8	2762.2
Ahero	1968.0	4721.0	3344.5	2731.0	4190.8	3460.9
Wkano	2079.8	5290.8	3685.3	2455.5	3357.0	2906.3
Bunyala	2111.8	4314.8	3213.3	1715.5	4929.5	3322.5
Taveta1	2154.3	3871.5	3012.9	2345.6	4366.0	3355.8
Taveta 2	1795.5	3755.5	2775.5	2216.5	3362.0	2789.3
TARDA 2	2277.0	4279.0	3278.0	3612.5	4572.3	4092.4
Means	3558.3	4255.8		3243.0	4062.6	
P-value P	0.0001			0.0001		
P-value accession	0.0001			0.0001		
P-value accession x P	0.0001			0.0001		
LSD (0.05) P	110.8			123.7		
LSD (0.05) accession	207.3			231.4		
LSD (0.05) accession x P	319.0			285.7		
CV (%)	7.2			6.2		

Effect of interaction between accession, P and N on Azolla tissue P and N

The effect of interaction between P and N on tissue P was significant during both seasons. However, it was not significant on tissue N during the same period (Table 10). During both first and second season treatment with 0 and 200mg N l⁻¹ significantly increased tissue N in both 0 and 3mg P l⁻¹ treatments. Application of 3mg l⁻¹ P also significantly increased tissue P in both seasons. The interaction between N and P on tissue P significantly increased tissue P in both seasons.

Table 10. Effect of interaction between P and N on *Azolla* tissue N and P.

	Season 1					
	Tissue N (%)			Tissue P (ppm)		
	0mg N l⁻¹	200mg N l⁻¹	mean	0mg N l⁻¹	200mg N l⁻¹	Mean
0mg P l⁻¹	3.1	4.6	3.85	1911.9	2033.2	1972.6
3mg P l⁻¹	3.4	4.8	4.1	3921	4590.6	4255.8
Mean	3.25	4.7		2916.45	3311.9	
P-value N	0.0001					
P-value P				0.0001		
P-value P x N	0.99			0.0001		
LSD (0.05) N	0.1					
LSD (0.05) P				110.8		
LSD (0.05) P x N	NS			170.5		
CV (%)	7.2			7.2		
	Season 2					
0mg P l⁻¹	2.8	4.0	3.4	2279.9	2560.0	2420.0
3mg P l⁻¹	3.1	4.4	3.8	4061.6	4063.6	4062.6
Mean	3.0	4.2		3170.8	3311.8	
P-value N	0.0001					
P-value P				0.0001		
P-value P x N	0.240			0.016		
LSD (0.05) N	0.1					
LSD (0.05) P				123.7		
LSD (0.05) P x N	NS			11.3		
CV (%)	4.8			6.2		

Discussion

The average temperature during *Azolla* biomass growth averaged 20.8°C and 21.9°C during the first and second seasons respectively. High temperatures in Mwea Irrigation Scheme are therefore conducive for *Azolla* growth and biomass accumulation, which may be beneficial for increased soil nutrient status. Reported findings showed that *Azolla* tolerates wider temperature but with an optimum of 23°C (http/theazollafoundation.org).

Irrigation water from all the schemes had a near to neutral pH with a range of 6.9 to 7.5. Ahero and Bunyala irrigation water had significantly higher pH than others sampled areas. The pH range was within the suitable range of 4.5-7, which is best for maximum growth of *Azolla* biomass. According to Wong *et al.* (1987), the best pH for optimum *Azolla* growth is 4.5. The relatively high pH in Ahero and Bunyala irrigation water samples suggests low contamination with fertilizers, which is one major source of acidity.

The tissue nitrogen levels for *Azolla* accessions ranged from 3.1% and 5.1%. It was significantly high in Ahero and low in Taveta 1 accessions. The levels were in concurrence with reported finding of 4-5% N by Watanabe (1983). Azolla tissue N showed a corresponding increase with increase in irrigation water N levels. Ahero and West Kano accessions had significantly the highest levels of N and similarly their irrigation water had relatively high levels of N. This suggests that flood water nutrients influenced *Azolla* tissues N. Sah *et al.* (1989) reported an increase in *Azolla* tissue N and P with increase in media water N and P.

The nutrient P is important for *Azolla* growth (Sudhi *et al.*, 1981). The tissue P ranged from 0.2-0.45% (1999-4495ppm) and also within the reported findings of 0.1-.0.5% (Better Crops, 1999). However, the negative correlation between media P and tissue P contradicts findings by Sah *et al.* (1989), which reported a positive relationship. However, it is in conformity with reported findings by Subudhi and Watanabe (2012) of a depressed tissue P levels with media P beyond 5ppm. The irrigation water P levels of 16ppm and up to 28ppm P may have been excess and beyond threshold limits. Subudhi and Watanabe (2012) recommended 5ppm P as adequate amounts within the irrigation water.

Azolla accessions from different irrigation schemes in Kenya had varied biomass growth rates and doubling times. Doubling time ranged from 5.5 to 6.7 and was higher during the first than in the second season was affected by temperatures. The varied *Azolla* biomass growth rate and doubling time may be attributed to existence of different *Azolla* species in Kenya. The doubling rate range of 5.6 to 6.7 was within the reported findings of 3-10 days by Hussner (2010) and 7-10 days by Campbell (2011). The doubling rate was however higher than 2.2-3.4 days reported by Kitoh (1993), under laboratory conditions. The shorter doubling time of *Azolla* during the first season may have been partly contributed to by higher average temperatures, which may have resulted into increased physiological and enzymatic activities. The effect of temperatures on increasing enzymatic, stomatal conductance and photosynthetic activities of plants has previously been reported (Sage *et al*., 2007; Kondo *et al*., 1989).

The nutrient N significantly affected biomass growth and doubling rates. Application of 200mg N l^{-1} generally reduced biomass growth rate for most of the *Azolla* accessions. The reduction in *Azolla* biomass accumulation can be attributed to the inhibitory effect of N on Acetylene Reduction and Nitrogenase activity as reported by Wagner (1997) and Singh, (1992). Nitrogen has has also been reported reduce the frequency of the heterocysts, which are important sites for N fixations (Singh, 1989). External nitrogen source therefore hinders *Azolla* biomass growth (Sadeghi, 2013). However, levels below 5nM have been reported to promote *Azolla* growth (Singh, 1992). This therefore suggests that application of 200mg l^{-1} N was way above the threshold limits for most *Azolla* growth. However, increase in biomass growth for Mwea, Taveta 2 and TARDA 2 accession may be due to their abilities to tolerance high N levels. Varied N tolerance amongst *Azolla* species has been reported (Singh, 1989). This therefore suggests that amongst the accessions used, there may have been different species or different ecological adaptations in environments of high N fertilizer usage.

Application of nutrient P generally reduced biomass growth and increased doubling time for *Azolla* accessions. This may be attributed to a negative effect of excess P on *Azolla* biomass growth. It suggests that P levels in canal water may have been more than adequate and that any addition was excess and above threshold limit requirements. The threshold limit of P is varied but 0.3ppm has been recommended (Subudhi & Watanabe, 2012). Yatazawa *et al.* (1980) recommended 0.03mmol 1^{-1} which is less than the treatment amounts. Findings by Pitt *et al.* (2014) showed that external sources of phosphorus can enhance growth of *Azolla* but excess P has negative effects on biomass growth. The amounts should therefore be within the required levels for appropriate growth (Kitoh, 1993; Sadeghi, 2012a; Costa, 1999).

Levels of P in the canal water were also found to be 0.8mgkg^{-1}, which was within the threshold limits and hence the higher biomass growth observed. Treatment with P levels of 3mg P l^{-1} may therefore have been beyond the requirement causing a negative effect on biomass growth.

Conclusion

The study therefore showed that *Azolla* accessions from different irrigation schemes in Kenya have varied tissue nutrient levels. It also showed that nutrient N and P affect biomass growth rate and that the levels of P in Mwea irrigation scheme is beyond threshold requirements for *Azolla* growth and multiplication. It also showed that media N increases tissue N but inhibits biomass growth.

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