Response of Highbush Blueberry to Nitrogen Fertilizer During Field Establishment, I: Accumulation and Allocation of Fertilizer Nitrogen and Biomass

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Abstract. The effects of nitrogen (N) fertilizer application on plant growth, N uptake, and biomass and N allocation in highbush blueberry (*Vaccinium corymbosum* L. 'Bluecrop') were determined during the first 2 years of field establishment. Plants were either grown without N fertilizer after planting (0N) or were fertilized with 50, 100, or 150 kg·ha^{-1} of N (50N, 100N, 150N, respectively) per year using ^{15}N-depleted ammonium sulfate the first year (2002) and non-labeled ammonium sulfate the second year (2003) and were destructively harvested on 11 dates from Mar. 2002 to Jan. 2004. Application of 50N produced the most growth and yield among the N fertilizer treatments, whereas application of 100N and 150N reduced total plant dry weight (DW) and relative uptake of N fertilizer and resulted in 17% to 55% plant mortality. By the end of the first growing season in Oct. 2002, plants fertilized with 50N, 100N, and 150N recovered 17%, 10%, and 3% of the total N applied, respectively. The top-to-root DW ratio was 1.2, 1.6, 2.1, and 1.5 for the 0N, 50N, 100N, and 150N treatments, respectively. By Feb. 2003, 0N plants gained 1.6 g/plant of N from soil and pre-plant N sources, whereas fertilized plants accumulated only 0.9 g/plant of N from these sources and took up an average of 1.4 g/plant of N from the fertilizer. In Year 2, total N and dry matter increased from harvest to dormancy in 0N plants but decreased in N-fertilized plants. Plants grown with 0N also allocated less biomass to leaves and fruit than fertilized plants and therefore lost less DW and N during leaf abscission, pruning, and fruit harvest. Consequently, by Jan. 2004, there was little difference in DW between 0N and 50N treatments; however, as a result of lower N concentrations, 0N plants accumulated only 3.6 g/plant (9.6 kg·ha^{-1}) of N, whereas plants fertilized with 50N accumulated 6.4 g/plant (17.8 kg·ha^{-1}), 20% of which came from ^{15}N fertilizer applied in 2002. Although fertilizer N applied in 2002 was diluted by non-labeled N applications the next year, total N derived from the fertilizer (NDFF) almost doubled during the second season, before post-harvest losses brought it back to the starting point.

Highbush blueberry (*Vaccinium* sp.) area in North America has increased from 27,105 ha in 2003 to 38,690 ha in 2008, a rate of 2,317 ha/year (Brazelton, 2009; Strik and Yarborough, 2005). Blueberry production worldwide has increased 82% in the same time period to 65,990 ha in 2008, a planting rate of 5,950 ha/year (Brazelton, 2009; Brazelton and Strik, 2007). To these fields, growers have generally been applying recommended rates of N fertilizer, 55 kg·ha^{-1} for newly established fields to 110 kg·ha^{-1} for mature plantings at 1.2 m × 3 m spacing (Hart et al., 2006; Pritts et al., 1992; Strik et al., 1993; Strik and Hart, 1992) or have been applying much higher rates, depending on production practices (Yang, 2002; Strik, personal observation).

Various studies have been completed on the effect of rate of N fertilization on the growth and yield of mature blueberry plants. In a 5-year study on 'Bluecrop' blueberry, plants fertilized with a split application of 75 kg·ha^{-1} of N had higher yields than unfertilized controls (Hanson and Retamales, 1992). In contrast, Clark et al. (1998) found that yield of 'Collins' blueberry was unaffected by fertilization rates of 22 and 112 kg·ha^{-1} of N, and in fact, applications of 67 and 134 kg·ha^{-1} of N reduced yield compared with 22 kg·ha^{-1} of N over 2 years. In an 8-year study, Cummings (1978) found that yield was not increased at N fertilization rates above the lowest rate of 34 kg·ha^{-1}.

Throop and Hanson (1997) showed that mature blueberry plants absorbed fertilizer N most efficiently during active growth between late bloom and fruit maturity; however, only 8% of the fertilizer was recovered 2 weeks after application. Mature 'Bluecrop' plants fertilized with 40 kg·ha^{-1} of N, as urea, before budbreak recovered a much greater proportion (32%) of the fertilizer N by the next fall (Retamales and Hanson, 1989). These studies suggest that multiple applications of fertilizer N are necessary to maintain sufficient soil N levels throughout the active growth period of blueberry.

No work has been published to date on N fertilizer uptake and partitioning in young, field-grown blueberry plants. The objectives of our study were to determine the impact of N fertilizer application on N uptake, allocation, and growth of young, field-grown blueberry plants during the first 2 years of establishment.

Materials and Methods

A planting of 'Bluecrop' blueberry was established on 27 Mar. 2002 at the North Willamette Research and Extension Center in Aurora, OR (long. 45°17′ N, lat. 122°45′ W; 46 m above sea level; last spring freeze occurs on 17 Apr., on average, and first fall freeze occurs on 25 Oct.). Plants were spaced at 1.2 m in the row with 3.0 m between rows (2777 plants/ha) on Quatama series soil (fine-loamy, mixed, mesic Aqualtic Haploxeralfs). Douglas fir (*Pseudotsuga menziesii* Franco) sawdust, 0.2 m deep and 0.3 m wide centered on planting rows, and fertilizer (66 kg·ha^{-1} of N as ammonium sulfate), a standard commercial practice when adding fresh organic matter, were incorporated in the soil before planting 2-year-old container-grown stock (4-L pots). No surface sawdust mulch was applied after planting. Flower buds were pruned off the plants at planting to prevent fruit production in the first growing season (2002). Plants were pruned in Winter 2002–2003 per standard commercial practice and prunings removed from the field.

The fertilizer treatments after planting were: no additional fertilizer N (0N) in 2002–2003; and three rates of N, 18.6, 37.2, or 55.8 g/plant per year (equivalent to 50, 100, or 150 kg N/ha, 50N, 100N, 150N, respectively) in 2002 and 2003. The total rate of N was divided into thirds and applied on 11 Apr., 20 May, and 27 June 2002 of the planting year and on 24 Mar., 8 May, and 23 June 2003 in Year 2 at the

same rate. Labeled ^{15}N-depleted ammonium sulfate [$(NH_4)_2SO_4$] was applied in 2002 and unlabeled ammonium sulfate was applied in 2003. Fertilizer was broadcast on the soil surface under the plant canopy in a circular area. All treated plots were fertilized with 35 kg·ha^{-1} of phosphorus as triple superphosphate and 66 kg·ha^{-1} of potassium as potassium sulfate each spring. Plants were irrigated with an overhead system and were otherwise maintained according to standard commercial practice (Strik et al., 1993).

The experimental treatments were arranged in a randomized complete block design with three replicates. The experimental unit consisted of a plot of 12 plants. One plant per plot was randomly selected and destructively harvested on each of 11 dates: 27 Mar., 29 Apr., 7 June, 24 July, and 29 Oct. 2002; 7 Feb., 22 Apr., 22 May, 25 July, and 27 Oct. 2003; and 8 Jan. 2004. Plants were dug from the field to a depth of 0.30–0.45 m to recover as much of the root system as possible. Roots were washed with high-pressure water to remove soil, and plants were separated into the following parts: flowers and fruit (2003 only), new shoots, leaves (removed from shoots), woody canes, crown, and roots. Leaves collected in late October of each year were considered to be at or near time of abscission based on field observations. In this study, plants dug in late October were considered to be at the end of the growing season.

Each part was oven-dried at 70 °C, to a constant weight, and dry weight was measured. A subsample of each tissue was ground to pass a 40-mesh screen (0.42-mm openings) and measured for total N and ^{15}N concentrations by mass spectrometry (Isotope Service, Los Alamos, NM).

Soil samples were collected from the potting medium of nursery plants before planting and the rooting zone of harvested plants on each date at a depth of ≈0.15–0.40 m. Soil samples were analyzed for pH, total N, NH_4^+, NO_3^-, and ^{15}N concentrations (Central Analytical Laboratory, Oregon State Univ., Corvallis, OR) from Sept. 2002 through Jan. 2004, except for ^{15}N for which samples ended May 2003.

Atom percent values were converted to the proportion of NDFF using standard conversions (Hauck and Bremner, 1976): %NDFF = [(^{15}N natural abundance) – (atom % [15]N$_{sample}$)]/ [(^{15}N natural abundance) – (atom % [15]N$_{fertilizer}$)] × 100. The ^{15}N natural abundance was assumed equal to 0.366 atom percent.

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The effect of N fertilizer rate and sampling date on each measured variable was analyzed using the PROC MIXED procedure in SAS Version 9.1 (SAS Institute Inc., Cary, NC). Partitioning of DW and N among plant parts was expressed as a percentage of the plant total and arcsine transformed before analysis. Back-transformed values are presented in figures and tables. Plant response to N fertilizer application rate was determined using orthogonal contrasts and pairwise comparisons.

Results and Discussion

Plant growth. Nitrogen fertilizer application rate (N rate) significantly affected plant DW over time ($P < 0.0001$) (Fig. 1A). In the first growing season, total DW increased rapidly from budbreak in April to onset of leaf abscission in Oct. 2002. Plants fertilized with 50N had the greatest DW at the end of the first growing season (0.36 kg/plant), whereas 150N plants had the least growth.

Plants fertilized with 100N and 150N showed symptoms of salt stress (e.g., interveinal chlorosis and marginal leaf necrosis in young developing leaves; Caruso and Ramsdell, 1995) in Year 1 with 17% and 55% of the plants dying by Oct. 2002, respectively. Cummings (1978) likewise observed 23% mortality in 4-year-old highbush blueberry plants fertilized with 168 kg·ha^{-1} of N in North Carolina. In both cases, ammonium-based fertilizers were used, which are often recommended for blueberry because 1) the plants prefer the NH_4^+ form of N over NO_3^-; and 2) the fertilizers are acidifying, and blueberry plants grow best when soil pH is
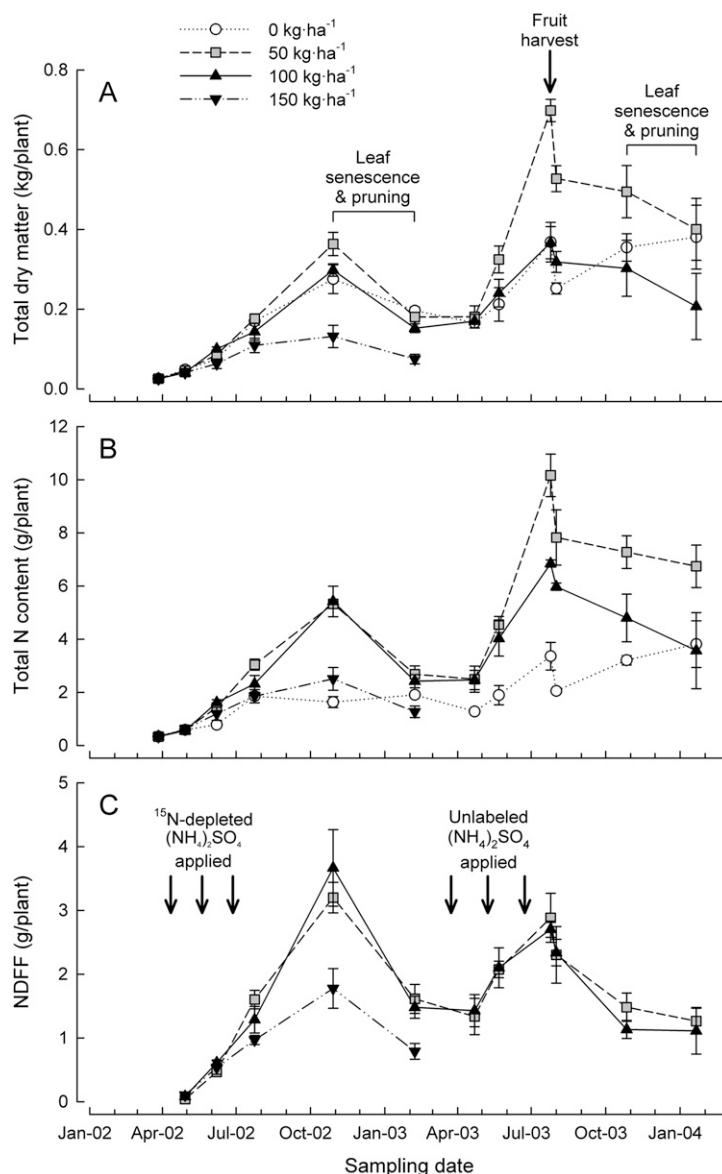

Fig. 1. Total dry matter (**A**), total nitrogen (N) content (**B**), and N derived from fertilizer (NDFF) (**C**) in young plants of 'Bluecrop' blueberry. Plants were established on 27 Mar. 2002 and left unfertilized after planting (0) or fertilized each spring with 50, 100, or 150 kg·ha^{-1} of N. Time of natural leaf senescence, manual pruning, and fruit removal indicated in **A** and fertilizer application indicated in **C**. NDFF represents labeled N applied during the first year after planting and does not include any N applied the next year. Each symbol represents the mean of three replicates and error bars represent ± 1 SE.

between 4.2 and 5.5 (Eck, 1988; Korcak, 1988; Poonnachit and Darnell, 2004); however, these fertilizers are very rapidly absorbed and also have a high salt index and therefore often lead to salinity problems in many plants, including blueberry, when overapplied (Ludwick, 1998). Bryla and Machado (2011) observed that electrical conductivity (EC) of soil solution under young blueberry plants reached levels as high as 4 dS·m^{-1} when ammonium sulfate was applied at a rate of 50 kg·ha^{-1} of N and up to 5–8 dS·m^{-1} when applied at a rate of 100–150 kg·ha^{-1} of N. In our study, we did not measure soil EC, but it is known that blueberry is considered sensitive to EC greater than 1.5–2.0 dS·m^{-1} (Patten et al., 1988). In contrast, young 'Star' southern highbush blueberry plants grown in pine bark had increased plant growth and yield with N rates as high as 50–81 g/plant or 360–580 kg·ha^{-1} of N (Williamson and Miller, 2009). In this case, plants were at a much higher density (7176 plants/ha) than in our study, and fertilizer was applied gradually every 4 weeks from April to August or every 2 weeks by fertigation. Plant-available N was also likely much lower in the pine bark culture system. In our study, plants that survived at the highest N rate in 2002 had essentially no growth in 2003.

The top-to-root DW ratio in Oct. 2002, at the end of the first growing season, was 1.2, 1.6, 2.1, and 1.5 for the 0N, 50N, 100N, and 150N treatments, respectively (data not shown). Unfertilized plants had a lower DW of leaves and shoots than those fertilized with 50N. Plants fertilized with 150N had a lower total plant DW as a result of less weight in all plant parts.

Over winter, total DW decreased from Oct. 2002 to Feb. 2003 as a result of leaf abscission and removal of wood during pruning (Fig. 1A). After pruning in Winter 2002–2003, there was no difference in plant DW among the 0N, 50N, and 100N treatments nor was there an effect on percent allocation of DW to plant parts. However, 0N and 50N plants had greater root DW (average of 99 g; $P < 0.0001$) in Feb. 2003 than 100N plants (82 g) or 150N plants (33 g). Plants fertilized with 50N had the greatest increase in DW in Year 2 from budbreak in Apr. 2003 to fruit harvest in July. Fruit DW at harvest averaged 0.12, 0.17, and 0.05 kg/plant for the 0N, 50N, and 100N treatments, respectively. In each treatment, plant DW reached a maximum at harvest. Plants fertilized with 50N had a total yield of 2.6 t·ha^{-1} of fresh fruit, a high yield for the second growing season, and greater than the yield of the 0N and 100N treatment plants (1.9 and 0.8 t·ha^{-1}, respectively). Higher rates of N fertilization have also reduced yield of young plants in other studies (Cummings, 1978).

By the end of the second growing season, differences in total DW among treatments were reduced after leaf abscission in late October, although plants fertilized with 50N still had significantly more total DW than the 0N and 100N plants ($P < 0.0001$); however, there was no difference in DW or allocation

of DW among plant parts between the 50N and 0N treatments by the beginning of Year 3, mostly a result of less pruning or wood removal in plants grown without additional fertilizer (0N; data not shown). There was a trend ($P = 0.08$) for 100N plants to have less root DW (73 g) than 0N (158 g) or 50N (151 g) plants in Jan. 2003.

Biomass per hectare (2777 plants/ha) in plants fertilized with 50N increased from 71 kg·ha^{-1} in Mar. 2002 to a maximum of 1940 kg·ha^{-1} in July 2003 and a final biomass of 1112 kg·ha^{-1} DW by the end of the study in Jan. 2004. In comparison, 0N plants had a final biomass of 1057 kg·ha^{-1} DW (Fig. 1A).

In 0N plants, net biomass accumulation from planting to the beginning of the second growing season (after pruning in Feb. 2003) was 474 kg·ha^{-1} with losses of 119 kg·ha^{-1} in senescing leaves and 101 kg·ha^{-1} in prunings. In plants fertilized with 50N, net biomass accumulation was 430 kg·ha^{-1} with losses of 255 and 254 kg·ha^{-1} in senescing leaves and prunings, respectively. Plants fertilized with 50N or 100N had a similar gain in biomass to 0N plants but had greater losses in DW in 2002. The scenario was very similar the next season when 0N plants accumulated 512 kg·ha^{-1} DW from Feb. 2003 to Jan. 2004 and lost only 323, 158, and 281 kg·ha^{-1} in

Table 1. Dry weight allocation among different parts of young 'Bluecrop' blueberries during the first 2 years after planting.[z]

Sample date	N fertilizer (kg·ha^{-1})	Leaves	New shoots	Flowers/fruit	Woody canes 1 year old	2 years old	3 years old	Crown	Roots
27 Mar. 2002	—	—	—	—	65.5	—	—	—	34.5
29 Apr. 2002	0	—	8.0	—	45.3	—	—	7.9	38.8
	50	—	8.7	—	38.6	—	—	7.8	44.8
	100	—	11.5	—	51.0	—	—	11.8	25.7
	150	—	6.7	—	41.3	—	—	8.9	43.0
	Significance[w]		NS		NS			NS	NS
7 June 2002	0	18.3	5.4	—	20.4	—	—	6.6	49.3
	50	20.1	3.8	—	24.7	—	—	5.5	45.9
	100	19.1	4.4	—	21.7	—	—	7.9	46.8
	150	16.7	2.8	—	27.0	—	—	5.6	47.8
	Significance	NS	NS		NS			NS	NS
24 July 2002	0	24.3	8.3	—	19.1	—	—	7.6	40.7
	50	31.3	10.5	—	10.2	—	—	3.1	45.0
	100	23.7	7.7	—	15.7	—	—	5.6	47.3
	150	17.0	7.1	—	20.5	—	—	8.2	47.1
	Significance	NS	NS		Q**			Q**C*	NS
29 Oct. 2002	0	15.3	14.1	—	14.4	—	—	10.6	45.6
	50	24.9	17.4	—	10.3	—	—	9.5	37.9
	100	28.8	15.9	—	13.0	—	—	9.8	32.5
	150	22.4	11.3	—	20.0	—	—	7.1	39.3
	Significance	L*Q**	NS		NS			L**	NS
7 Feb. 2003	0	—	—	—	13.1	21.9	—	13.8	51.2
	50	—	—	—	18.9	14.9	—	11.7	54.5
	100	—	—	—	21.1	13.8	—	11.2	53.9
	150	—	—	—	12.0	30.4	—	13.9	43.7
	Significance				NS	NS		NS	NS
22 Apr. 2003[x]	0	—	2.0	2.5	13.2	11.9	—	21.6	48.9
	50	—	4.2	2.1	14.6	13.0	—	15.9	50.2
	100	—	3.3	2.0	11.7	16.8	—	16.7	49.5
	Significance		NS	NS	NS	NS		NS	NS
22 May 2003	0	6.1	1.2	6.1	14.8	13.7	—	11.6	46.7
	50	14.5	2.6	4.0	15.4	9.3	—	7.1	47.1
	100	13.4	2.4	3.4	21.4	9.5	—	9.0	40.9
	Significance	L**Q*	L*	NS	NS	NS		Q*	NS
27 July 2003	0	10.6	4.5	29.8	10.9	7.5	—	10.7	26.0
	50	17.6	5.2	24.5	9.3	8.3	—	7.4	27.8
	100	26.1	6.8	12.7	8.7	8.5	—	11.0	26.1
	Significance	NS	NS	NS	NS	NS		NS	NS
27 Oct. 2003	0	16.3	11.4	—	12.2	13.5	—	19.9	26.6
	50	28.9	20.2	—	12.1	7.1	—	12.3	19.4
	100	29.9	19.9	—	9.7	6.1	—	12.5	21.9
	Significance	L*	L**Q*		NS	L**		L**Q*	NS
21 Jan. 2004	0	—	—	—	13.2	14.4	17.6	13.8	41.0
	50	—	—	—	16.8	14.2	16.8	11.5	40.7
	100	—	—	—	23.1	12.7	13.8	11.7	38.8
	Significance				NS	NS	NS	NS	NS

[z]Plants were established on 27 Mar. 2002 and fertilized with nitrogen each spring.[y]
[y]Plants were leaf unfertilized after planting (0) or were fertilized with 50, 100, or 150 kg·ha^{-1} of N in spring of each year.
[x]Most plants fertilized with 150 kg·ha^{-1} of N died by 22 Apr. 2003.
[w]NS = nonsignificant; L = linear; Q = quadratic; or C = cubic response at *$P < 0.05$ or **0.01 based on orthogonal contrasts.
N = nitrogen.

fruit, senescing leaves, and prunings, respectively, whereas 50N plants accumulated 611 kg·ha^{-1} DW and lost 475, 389, and 483 kg·ha^{-1}, respectively. Clearly, 0N plants allocated similar amounts of biomass to new canes, crown, and roots but much less biomass to leaves and fruit, as did 50N or 100N plants.

Allocation of DW varied with sample date and N rate (Table 1). Increasing rate of N fertilizer application increased DW allocation to leaves in the fall of 2002 and to leaves and new shoots in the spring and fall of 2003 but had no impact on allocation to flowers and fruit. Retamales and Hanson (1989) reported fruit DW accounted for 3.7% of total DW in fall on mature plants. In our study, on these young plants, fruit would have accounted for 17%, 33%, and 35% of total plant DW in Oct. 2003 for the 100N, 50N, and 0N treatments, respectively, if fruit were included at this stage of development. Dry weight allocation to wood also responded significantly to N fertilization rate, but only in 1-year wood in July of 2002, when DW allocation to wood was lowest in 50N plants, and in 2-year wood in Oct. 2003, when less DW was allocated to the wood as more N fertilizer was applied (Table 1). Belowground, plants often allocated less DW to crown tissue at higher N application rates, but there was little difference in the proportion of DW allocated to roots. Roots accounted for 35% of total plant DW at planting and, with the exception of plants fertilized with 150N, maximum allocation of DW to roots, 53% on average, occurred in Feb. 2003. Crown and root tissue accounted for 51% to 55% of total plant DW at the end of this study, similar to the 50% reported by others for mature blueberry plants (Pritts and Hancock, 1985; Retamales and Hanson, 1989).

Nitrogen uptake and partitioning. Total plant N content, which averaged 0.34 g at planting, was significantly affected by N rate over time ($P < 0.0001$) (Fig. 1B). Plants fertilized with 50N accumulated more N by Oct. 2002 than 0N or 150N plants and more N than all treatments by fruit harvest in 2003. Plants fertilized with 50N accumulated more than threefold the total N as 0N plants in Oct. 2002, but by Feb. 2003, the total N content of 50N plants was only 40% greater than that of 0N plants as a result of greater losses of N over the winter of 2002–2003. By the end of the study in Jan. 2004, plants fertilized with 50N gained a total of 17.8 kg·ha^{-1} of N and lost 6.5 kg·ha^{-1} of N in fruit and 13.3 kg·ha^{-1} of N in senescing leaves, whereas 100N plants gained only 9.2 kg·ha^{-1} of N and lost 2.5 kg·ha^{-1} of N in fruit and 9.7 kg·ha^{-1} of N in senescing leaves. The 0N plants gained 9.9 kg·ha^{-1} of N, similar to 100N plants, but lost 3.6 kg·ha^{-1} of N in fruit and 2.3 kg·ha^{-1} of N in senescing leaves, much less than that found in the 50N and 100N plants. Nitrogen was also lost by removal of wood when pruning each winter; the 0N plants lost a total of 2.2 kg·ha^{-1} of N, whereas 50N and 100N plants lost 6.9 and 1.9 kg·ha^{-1} of N, respectively. Note that any N in abscised leaves and prunings that are chopped and left in the field

(as is common commercial practice) would likely return to the plant/soil system as materials decompose. Nitrogen recovery from leaves and flailed prunings averaged 9% over 2 years in kiwifruit (*Actinidia deliciosa* A. Cher.; Ledgard et al., 1992) and 8% over 1.5 years in red raspberry (*Rubus idaeus* L.; Strik et al., 2006).

Nitrogen fertilization rate also affected allocation of total N within plants (Table 2). Increasing rate of N fertilizer application increased allocation of N to leaves from July to leaf senescence in October, but allocation of N to the crown and roots declined with increasing N rate on many dates. Plants also

generally allocated more N to woody canes with higher N rate, although results varied with wood age and sample date. The proportion of total plant N present in the leaves in fall in the young fertilized plants (41% to 48% in 2002 and 20% to 36% in 2003) was higher than that reported for mature plants (17%; Retamales and Hanson, 1989), suggesting that relative allocation of total N to leaves may decrease in older plantings. In Jan. 2004, allocation of N among canes, crown, and roots was similar between fertilized (50N and 100N) and 0N plants (Table 2).

With few exceptions, N concentration in each plant part was lowest in 0N plants and

Table 2. Nitrogen (N) allocation among different parts of young 'Bluecrop' blueberries during the first 2 years after planting.[z]

| | | | | | N allocation (%) | | | | |
| | | | | | Woody canes | | | | |
Sample date	N fertilizer (kg·ha^{-1})	Leaves	New shoots	Flowers/fruit	1 year old	2 years old	3 years old	Crown	Roots
27 Mar. 2002	—	—	—	—	59.2	—	—	—	40.8
29 Apr. 2002	0	—	20.3	—	39.9	—	—	9.7	30.1
	50	—	22.4	—	33.4	—	—	7.6	36.6
	100	—	27.5	—	37.0	—	—	11.5	23.9
	150	—	13.9	—	32.5	—	—	7.4	46.2
	Significance[w]		NS		NS			NS	NS
7 June 2002	0	33.0	5.2	—	10.3	—	—	6.7	44.8
	50	32.5	3.1	—	16.6	—	—	5.5	42.3
	100	34.2	3.8	—	14.5	—	—	7.4	40.1
	150	24.2	2.5	—	20.5	—	—	5.5	47.4
	Significance	NS	NS	—	L**C*			NS	NS
24 July 2002	0	31.5	5.1	—	6.6	—	—	3.4	53.5
	50	50.6	8.3	—	7.1	—	—	2.2	31.8
	100	40.5	7.6	—	12.8	—	—	5.7	33.4
	150	35.3	7.4	—	16.5	—	—	8.1	32.8
	Significance	NS	NS	—	L**			L**	L*Q*
29 Oct. 2002	0	13.9	15.6	—	12.4	—	—	11.2	46.8
	50	40.7	14.0	—	8.6	—	—	8.1	28.7
	100	47.7	12.7	—	9.9	—	—	7.5	22.2
	150	42.0	11.3	—	17.1	—	—	7.3	22.3
	Significance	L**Q**	L**		Q*			L**	L**Q*
7 Feb. 2003	0	—	—	—	10.1	12.5	—	15.5	61.9
	50	—	—	—	22.0	16.1	—	14.3	47.6
	100	—	—	—	27.6	15.4	—	12.3	44.7
	150	—	—	—	20.2	34.3	—	13.9	31.6
	Significance				NS	NS		NS	L**
22 Apr. 2003[x]	0	—	9.0	10.5	8.6	5.9	—	21.6	44.3
	50	—	14.4	7.5	13.9	11.9	—	17.2	35.2
	100	—	10.4	7.0	11.4	14.3	—	18.8	38.1
	Significance		NS	NS	NS	L**		NS	NS
22 May 2003	0	18.5	1.8	17.1	9.2	5.2	—	8.1	40.2
	50	30.0	2.7	8.7	10.9	5.6	—	4.8	37.3
	100	26.7	2.8	7.3	16.9	6.3	—	7.3	32.7
	Significance	L*Q*	L*	L*	NS	NS		NS	NS
27 July 2003	0	18.6	3.3	35.3	5.8	4.5	—	7.5	24.8
	50	26.9	3.7	23.7	5.1	4.5	—	5.7	30.5
	100	35.8	4.8	12.6	4.5	3.9	—	9.1	29.3
	Significance	NS	NS	NS	NS	NS		NS	NS
27 Oct. 2003	0	19.7	13.2	—	9.0	8.3	—	18.4	31.3
	50	35.9	14.3	—	8.7	4.9	—	10.8	25.4
	100	20.3	28.1	—	7.4	4.1	—	10.5	29.5
	Significance	Q**	L*		NS	NS		NS	NS
21 Jan. 2004	0	—	—	—	12.2	10.4	10.4	12.4	54.5
	50	—	—	—	15.9	11.5	10.0	9.5	53.0
	100	—	—	—	21.0	10.1	8.7	9.5	50.6
	Significance				NS	NS	NS	NS	NS

[z]Plants were established on 27 Mar. 2002 and fertilized with N each spring.[y]
[y]Plants were leaf unfertilized after planting (0) or were fertilized with 50, 100, or 150 kg·ha^{-1} of N in spring of each year.
[x]Most plants fertilized with 150 kg·ha^{-1} of N died by 22 Apr. 2003.
[w]NS = nonsignificant; L = linear; Q = quadratic; or C = cubic response at *$P < 0.05$ or **0.01 based on orthogonal contrasts.

often increased significantly as more N fertilizer was applied (Table 3). Based on nutrient guidelines for blueberry (Hart et al., 2006), leaf N concentration in late July was deficient (less than 1.5%) each year in 0N plants and above normal (greater than 2.0%) in all fertilized plants (Table 3). Leaf N concentrations in this study were from a subsample of all leaf tissue on each sample date and therefore may have underestimated N concentration in the most recent fully expanded leaves on which nutrient standards are based. It is possible that current leaf N standards, generally used for producing plants, may need to be increased for young plants.

Leaf N concentration declined from early spring through fall, as found by others (Bailey et al., 1962; Cheng and Raba, 2009; Ledgard and Smith, 1992; Retamales and Hanson, 1990) with the exception of 150N plants, which had high leaf N concentration and symptoms of salt stress and poor growth (Table 3). Flower and fruit N concentration also declined from flowering to fruit harvest but was higher than the fruit N concentrations observed by Ballinger and Kushman (1966) and Bishop et al. (1971). The presence of immature and mature fruit on the July sample date likely overestimated fruit N concentration. Like mature plants (Retamales and Hanson, 1989), wood N concentration was inversely related to wood age with highest concentrations in new shoots and lowest in 3-year-old canes. Belowground, crown N concentration decreased each summer but only in 0N and 50N plants. Root N concentration, on the other hand, peaked in June or July the first year after planting and increased throughout the season the next year regardless of the amount of N applied.

Nitrogen fertilizer recovery. Plant NDFF was affected by N rate ($P < 0.01$) and by sampling date ($P < 0.0001$) (Fig. 1C). The 50N and 100N plants had more NDFF than the 150N plants. Fertilizer N was found in every plant part and allocation varied throughout the year (Fig. 2).

By Oct. 2002, plants recovered 17% (3.2 g) of the applied fertilizer when 50N was applied, 10% (3.6 g) when 100N was applied, and only 3% (1.7 g) when 150N was applied. These amounts accounted for 58% to 70% of the total N in the plants, depending on N rate. In the 50N plants, ≈79% was allocated aboveground to leaves, new shoots, and woody canes (Figs. 2A, C, and D); thus, like total DW and N content, fertilizer N declined sharply over winter as a result of leaf abscission and pruning. After pruning, in Feb. 2003, an average of 60% of the total N in fertilized plants was NDFF, considerably higher than the 6% found in mature plants by Retamales and Hanson (1989).

In the 50N plants, 40% of the NDFF remaining after pruning in 2002–2003 was in woody canes, 46% in the roots, and 14% was in the crown (Fig. 2D–F). This stored N was likely an important source of N for new plant growth, as documented in potted rabbiteye blueberry (*V. virgatum* Ait.) plants (Birkhold and Darnell, 1993). We could not

directly measure plant use of stored NDFF in the second year, because the content of labeled N in fertilized plants increased from Apr. to July 2003 (Fig. 1C); this likely reflected uptake of fertilizer N, applied the previous year, from soil and possibly recycled fertilizer N from abscised, decomposed leaves and turnover of fine roots. Fine roots contain high concentrations of N and turnover readily under unfavorable soil conditions in blueberry (Valenzuela-Estrada et al., 2008). However, at the beginning of this period in Apr. 2003, 63% of the total N in flowers and new shoots of 50N plants was likely from stored NDFF, because presumably little to no N uptake had yet occurred that early in the season (Fig. 2B–C).

Total N and NDFF stored in the roots, crown, and woody canes of 50N plants declined in Spring 2003 as total N and NDFF in new growth increased (Fig. 2A–F).

In 2003, most new uptake of ^{15}N from fertilizer applied in 2002 occurred in the roots (Fig. 2F). In July 2003, 25% of total fruit N at harvest, in plants fertilized with 50N, was NDFF applied in 2002; any remaining N would have come from soil sources, stored N in the original planting material, or unlabeled fertilizer applied the second year (Fig. 2B). In mature kiwifruit, results were similar with ≈60% of the N used for new growth and production being from remobilization of N stored within the vines and ≈40%

Table 3. Nitrogen (N) concentration in different parts of young 'Bluecrop' blueberries during the first 2 years after planting.[z]

					Woody canes				
Sample date	N fertilizer (kg·ha⁻¹)	Leaves	New shoots	Flowers/fruit	1 year old	2 years old	3 years old	Crown	Roots
27 Mar. 2002	—	—	—	—	1.16	—	—	—	1.58
29 Apr. 2002	0	—	3.08	—	1.08	—	—	1.49	0.95
	50	—	3.36	—	1.12	—	—	1.30	1.07
	100	—	3.41	—	1.02	—	—	1.35	1.32
	150	—	3.12	—	1.19	—	—	1.28	1.68
	Significance[w]		NS		NS			NS	NS
7 June 2002	0	1.92	1.00	—	0.54	—	—	1.10	0.96
	50	2.70	1.27	—	1.13	—	—	1.68	1.56
	100	2.87	1.40	—	1.07	—	—	1.52	1.38
	150	2.76	1.65	—	1.41	—	—	1.87	1.85
	Significance	L*	L**		L**C*			L*	L**C*
24 July 2002	0	1.42	0.66	—	0.37	—	—	0.48	1.44
	50	2.82	1.32	—	1.19	—	—	1.21	1.25
	100	2.81	1.59	—	1.33	—	—	1.65	1.11
	150	3.84	1.79	—	1.38	—	—	1.69	1.19
	Significance	L**	L**		L**Q**			L**	NS
29 Oct. 2002	0	0.54	0.67	—	0.53	—	—	0.63	0.61
	50	2.39	1.19	—	1.24	—	—	1.27	1.12
	100	3.01	1.47	—	1.40	—	—	1.39	1.28
	150	3.73	1.97	—	1.67	—	—	2.04	1.11
	Significance	L**Q*	L**		L**Q*			L**	L*Q*
7 Feb. 2003	0	—	—	—	0.78	0.56	—	1.07	1.18
	50	—	—	—	1.70	1.58	—	1.73	1.34
	100	—	—	—	2.07	1.77	—	1.72	1.32
	150	—	—	—	2.91	1.87	—	1.52	1.22
	Significance				L**	L**Q**		NS	NS
22 Apr. 2003[x]	0	—	3.51	3.26	0.51	0.38	—	0.76	0.70
	50	—	4.67	5.28	1.32	1.26	—	1.48	0.95
	100	—	4.74	5.05	1.40	1.23	—	1.59	1.11
	Significance		L**Q*	L*Q*	L**Q*	L**Q**		L**	L**
22 May 2003	0	2.77	1.43	2.52	0.56	0.35	—	0.64	0.77
	50	2.95	1.53	3.09	0.98	0.84	—	0.95	1.12
	100	3.34	2.02	3.53	1.30	1.09	—	1.34	1.34
	Significance	L*	L**	L**	L**	L**		L**	L**
27 July 2003	0	1.42	0.66	1.05	0.48	0.52	—	0.66	0.88
	50	2.23	1.05	1.37	0.81	0.76	—	1.14	1.60
	100	2.26	1.34	1.79	1.00	0.86	—	1.57	2.15
	Significance	NS	L**	L*	L*	NS		L*	L**
27 Oct. 2003	0	1.10	1.07	—	0.68	0.56	—	0.84	1.08
	50	1.85	1.06	—	1.05	1.05	—	1.32	1.94
	100	1.11	2.34	—	1.29	1.10	—	1.37	2.18
	Significance	Q**	L**Q*		L*	NS		NS	L**Q*
21 Jan. 2004	0	—	—	—	0.96	0.74	0.61	0.92	1.37
	50	—	—	—	1.61	1.41	1.03	1.43	2.30
	100	—	—	—	1.56	1.35	1.13	1.44	2.28
	Significance				L*	L**Q*	L**	L*	L**Q**

[z]Plants were established on 27 Mar. 2002 and fertilized with nitrogen each spring.[y]
[y]Plants were leaf unfertilized after planting (0) or were fertilized with 50, 100, or 150 kg·ha⁻¹ of N in spring of each year.
[x]Most plants fertilized with 150 kg·ha⁻¹ of N died by 22 Apr. 2003.
[w]NS = nonsignificant; L = linear; Q = quadratic; or C = cubic response at *$P < 0.05$ or **0.01 based on orthogonal contrasts.
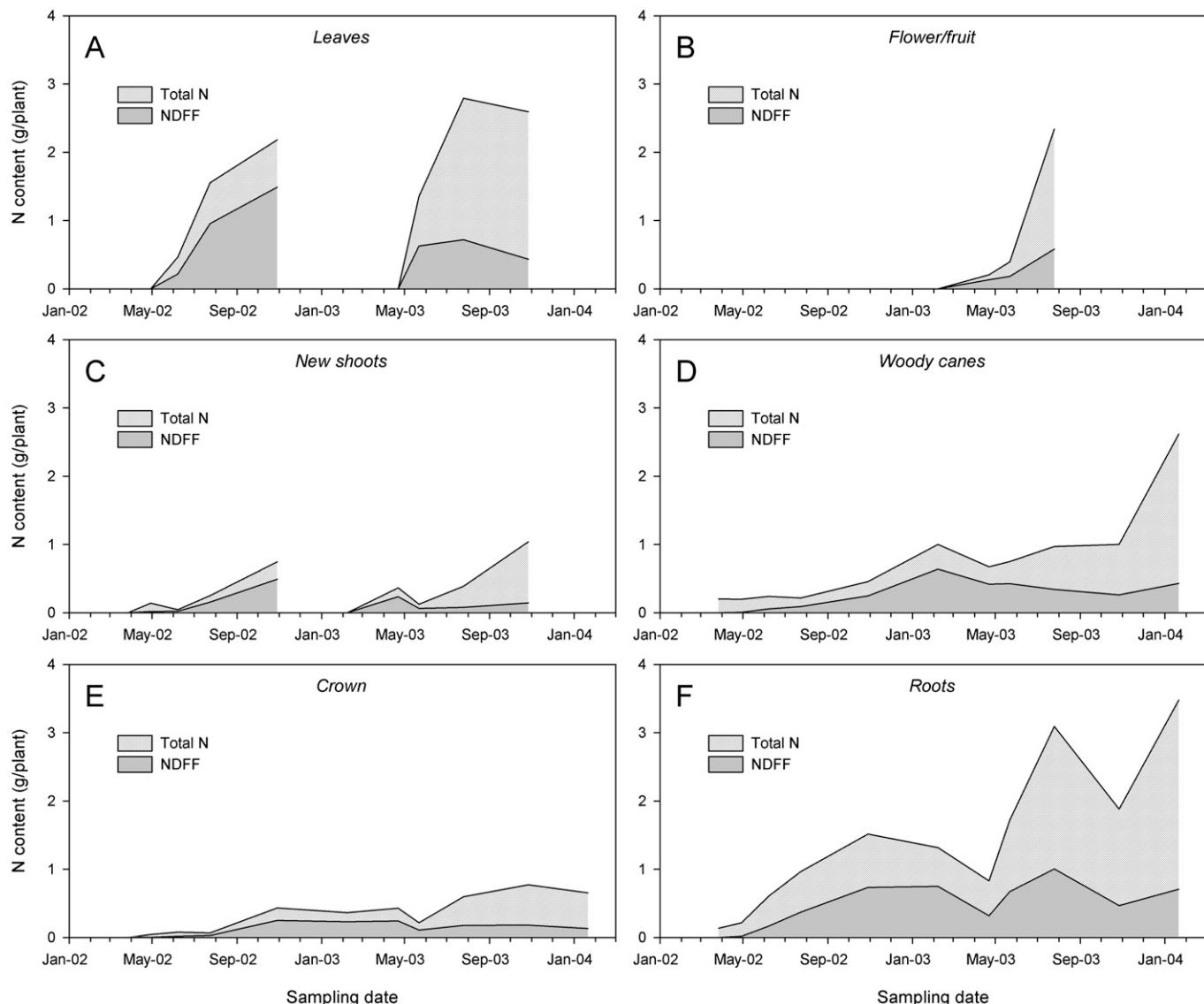
Fig. 2. Total nitrogen (N) and N derived from fertilizer (NDFF) in leaves (**A**), flowers and fruit (**B**), new shoots (**C**), woody canes (**D**), crown (**E**), and roots (**F**) of young plants of 'Bluecrop' blueberry. Plants were established in the field on 27 Mar. 2002 and fertilized each spring with 50 kg·ha^{-1} of N. NDFF represents labeled N applied during the first year after planting and does not include any N applied the next year. Time of fertilizer application is provided in Figure 1. Each symbol represents the mean of three replicates and error bars represent ± 1 SE.

from fertilizer and soil sources (Ledgard and Smith, 1992).

By the end of the second season in Oct. 2003, NDFF applied the first year accounted for 20% of the total N in plants fertilized with 50N and 24% of the total N in plants fertilized with 100N. By the end of the study, in Jan. 2004, 0N plants had accumulated 3.6 g of N (or 9.6 kg·ha^{-1}). The 50N plants accumulated 6.4 g of N, but only 20% of the accumulated N (1.3 g/plant) came from the fertilizer applied in 2002; the rest came from soil-available N or recycled N from abscised leaves or root turnover. Plants fertilized with 100N had 33% NDFF in Jan. 2004 but grew less and accumulated less N (3.3 g/plant) than those fertilized with 50N.

Soil pH and availability of soil nitrogen. Soil pH, availability of inorganic soil N, and soil NDFF all differed among N rates over time ($P < 0.0001$) (Fig. 3). Soil pH declined by 0.6–1.1 units with N fertilizer but was

similar regardless of the amount of N applied (Fig. 3A). Soil pH in 0N plots remained between 4.7 and 5.4 in both years, whereas soil pH in fertilized plots declined from an average of 4.7 in Apr. 2002 to ≈4.0 by the end of the year, a value considered somewhat low for highbush blueberry (Hart et al., 2006). In a 6-year study in Nova Scotia, Canada, yield was highest in unfertilized plants, likely because fertilization reduced soil pH below 4.0 (Thompson, 1973).

Soil NH_4^+ and NO_3^- levels in the nursery plant potting mix, at planting, averaged 2.2 and 0.1 mg·kg^{-1}, respectively. Soil NH_4^+ and NO_3^- levels increased as more N fertilizer was applied (Fig. 3B–C). In general, soil NH_4^+ increased rapidly after each N fertilizer application and then declined gradually over the growing season, whereas soil NO_3^- increased gradually until fall and then declined quickly over the winter rainy season. In 0N plots, soil NO_3^- levels remained low, only

increasing slightly in late spring/early summer the second year, as soil temperatures warmed up (Fig. 3C).

Even at the soil pH found in this study and with ammonium fertilizer applied, soil NO_3^- levels were relatively high, especially for the high rates of N fertilizer, in late summer/fall (Fig. 3C). Nitrification rates have been shown to be high in blueberry soils despite the low pH typically found (Hanson et al., 2002; Retamales and Hanson, 1990). Soil NO_3^- declined from fall to late winter in each year, because NO_3^- either moved beyond the soil sampling zone or was lost as a result of leaching. Soil NO_3^- also decreased over winter in Michigan (Retamales and Hanson, 1990).

Although we were not able to determine the total amount of fertilizer N remaining in the soil around the root zone of the blueberry plants, we did measure the proportion of the total N in the soil that was NDFF. Soil NDFF increased, as expected, with application of
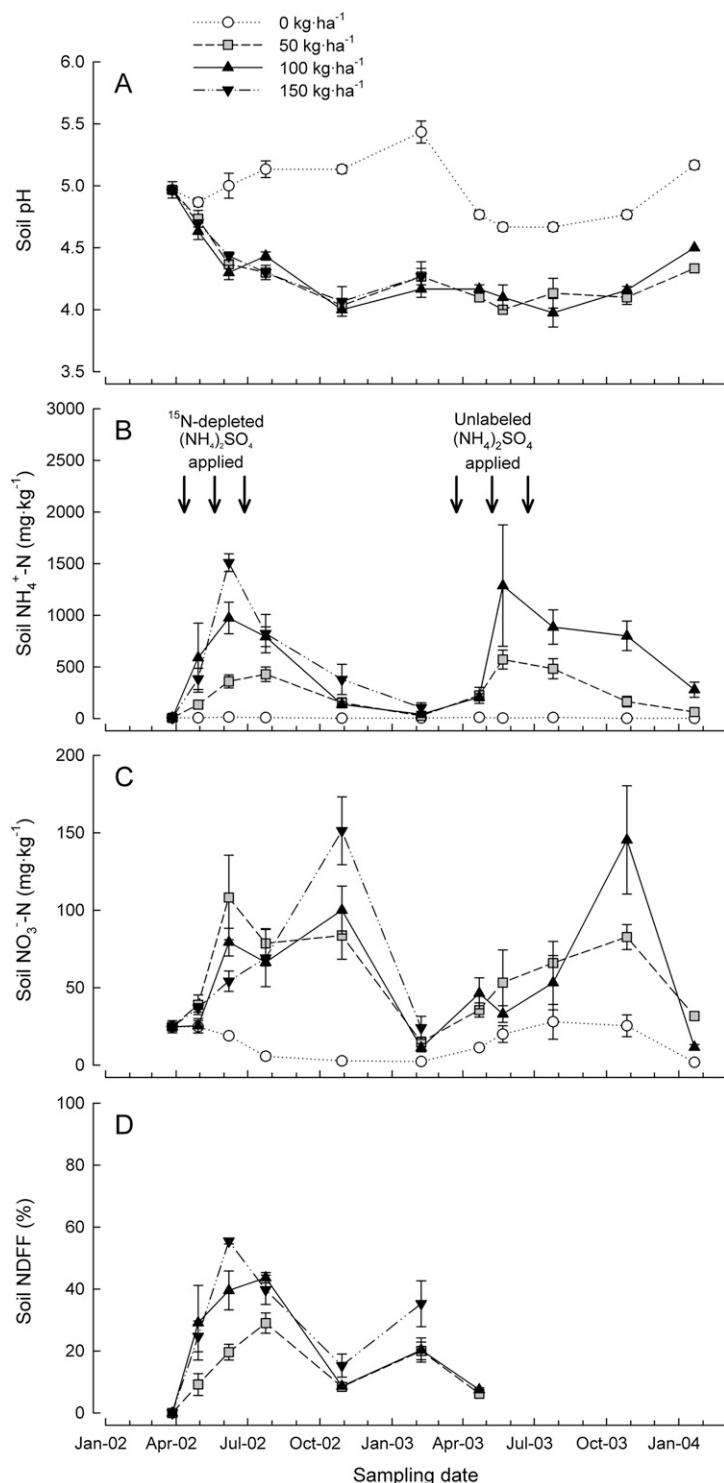
Fig. 3. Soil pH (**A**), NH$_4^+$-N (**B**), NO$_3^-$-N (**C**), and N derived from fertilizer (NDFF) (**D**) under young plants of 'Bluecrop' blueberry. Plants were established on 27 Mar. 2002 and left unfertilized after planting (0) or fertilized each spring with 50, 100, or 150 kg·ha^{-1} of N. Each symbol represents the mean of three replicates and error bars represent ± 1 SE. Arrows (**B**) denote times of N fertilizer application. Soil NH$_4^+$ and NO$_3^-$ levels in the nursery plant potting mix, at planting, averaged 2.2 and 0.1 mg·kg^{-1}, respectively. NDFF data were not available for soil samples taken after May 2003.

^{15}N-depleted fertilizer, peaking after the third application at 29% of the total soil N when 50N was applied and at 44% when 100N was applied but then declined in late summer, likely as a result of plant uptake (Fig. 3D). Approximately 10% to 18% of the total N in the soil samples in October was NDFF, similar

to the levels found in top soil under mature, fertilized blueberry plants at the end of the season in Michigan (Retamales and Hanson, 1989). Interestingly, over winter, from Oct. 2002 to Feb. 2003, soil NDFF level increased again, perhaps as a result of decomposition of leaf litter but also possibly from turnover of

fine roots (Valenzuela-Estrada et al., 2008). This increase over winter was then followed by another decline in soil NDFF in early spring (Fig. 3B) as a result of root uptake (Figs. 1C and 2F), although additional N fertilizer (unlabeled) was applied.

Conclusions

Application of 50 kg·ha^{-1} of N produced more growth and fruit production during the first 2 years after planting than either 100 or 150 kg·ha^{-1} of N as well as no N. This low rate of N, applied by hand around the drip line of each plant, was the best N fertilizer treatment at this planting density (2777 plants/ha); higher rates may be required in the establishment year for greater planting densities, until the plant canopies fill the in-row area. By the end of the first growing season, plants recovered 17% of the fertilizer N applied at 50 kg·ha^{-1} N, 10% applied at 100 kg·ha^{-1} N, and only 3% applied at 150 kg·ha^{-1} N. Additional fertilizer N applied the first season was taken up the next year, and by the beginning of the third season, 20% to 24% still remained in the plant.

During the first year after planting, plants grown without additional N fertilizer derived 100% of their N needs from four potential sources: pre-plant N (66 kg·ha^{-1} of N), plant N reserves, N available in the potting media at the time of transplanting, and N mineralized from the soil. Fertilized plants, in contrast, derived only 39% of their N from these sources and obtained the rest from N fertilizer applied at the beginning of the season. Interestingly, application of N fertilizer reduced N uptake from these alternative N sources by an average of 56%. Although it is possible that the unfertilized plants were growing in a less favorable higher soil pH than fertilized plants, the soil pH in the unfertilized treatment plots was still within the acceptable range for northern highbush blueberries (pH less than 5.5). Because blueberry plant response to N fertilization may also be a function of soil pH, ideally we would have had an acidified, unfertilized control in this study.

Plants fertilized with N also had higher N concentrations in each plant part and allocated more biomass to leaves and fruit than those grown without N fertilizer. As a result, fertilized plants lost up to nine times more N during leaf senescence and pruning and up to three times more N during fruit harvest than the unfertilized plants. By the end of the second season, total DW after pruning was similar between plants fertilized with 0 and 50 kg·ha^{-1} of N and lower in plants fertilized with 100 kg·ha^{-1} of N. Total N content, on the other hand, was similar between plants fertilized with 0 and 100 kg·ha^{-1} of N and higher in plants fertilized with 50 kg·ha^{-1} of N. It is clear in this study that excessive fertilization with N during establishment increases plant loss and reduces growth, likely as a result of ammonium toxicity or salt stress. If we had added a control that was fertilized with added salt (e.g., sodium) to EC levels similar to those produced at the higher rates of N fertilization,

we would have been able to more clearly study this aspect.

Next, we will determine the requirements of not only N in each plant part, but of other nutrients in the young plants and examine how the nutrient requirements were affected by the amount of N fertilizer applied. We will also examine uptake of fertilizer N and other nutrients in mature plants and eventually develop a complete nutrient budget for highbush blueberry.

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