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A Standardized RNA Isolation Protocol for Yam (*Dioscorea alata* L) cDNA Library Construction

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ABSTRACT

For the purpose of constructing yam cDNA libraries, attempts to isolate high quality RNA using several previously reported protocols were unsuccessful. Therefore a protocol was standardized for yam total RNA isolation by using guanidium buffer at the Department of Biology, Virginia Sate University. The RNA isolated using this standardized protocol was high in quality and led to successful good quality cDNA library construction and identification of functional ESTs in yam.

INTRODUCTION

Yam, (*Dioscorea alata* L), is the main food source for over 100 million people in humid and sub-humid tropics. Its production is affected by several biotic and abiotic factors (Abang et al., 2003). Anthracnose, caused by *Colletotrichum gloeosporioides*, is the most severe foliar disease of water yam (*Dioscorea alata* L) and is a major hurdle in yam production. It is reported that anthracnose causes yield reduction up to 90% (http://annualreport.iita.org). There are no cost effective control measures and the long-term solution to the problem will be through the development of resistant genotypes (Mignouna et al., 2002. Very limited yam sequence information is available from public genome databases. A review of previous efforts to develop cDNAs towards EST development in yams revealed that housekeeping genes were prevalent in the libraries constructed using total RNA from male flowers (Mignouna et al., 2002, b, c).

It is realized that obtaining high quality, intact RNA is the first and the most critical step in conducting cDNA library construction and for further analysis of gene of interest. After many attempts of total RNA isolations from yam leaf samples using standard plant RNA isolation protocols (Verwoerd et al,1989), only 6-10 ug of total RNA was extracted from the leaves and no colonies were observed when this RNA was used for cDNA library construction. The RNA appeared as a smear on 1.1% agarose gel (Fig. 1). The most likely reason for not getting good quality RNA is the mucilagenous tissue in yam plant parts like leaf, stem and tuber. This tissue causes problem because of polyphenols, polysaccharides and other secondary metabolites that are rich in yam plant parts and are not easily removed by conventional extraction methods. The aim of this study was to establish a protocol for RNA isolation from *Dioscorea alata* to get high quality and high quantity RNA that is suitable for generation of molecular markers, such as EST-SSRs and SNPs. Therefore, the following article discusses successful and reproducible method of RNA isolation



FIGURE 1. A smear of rRNA samples of Dm-Resistant yam genotype and Bm-Susceptible yam genotype isolated using standard protocols on 1.1% Formaldehyde agarose gel

procedure employed for yam cDNA library constrcution and ways of increasing RNA yields

MATERIALS AND METHODS

Tissue collection: In order to standardize the protocol for RNA isolation, the yam (source: local grocery store) were grown in the green house in pots. Fresh 1g leaf tissues are collected in 50ml BD Falcon tubes, frozen quickly in liquid nitrogen.

RNA isolation. Only the successful procedure of RNA isolation with the modifications to standard plant RNA isolation protocol is reported here.

Solutions and solvents used:

- Extraction buffer (100 ml stock): 76.424g of 8M Guanidium Hydrochloride + 425 mg of 20mM MES + 740mg of 20mMEDTA+ 35ml of DEPC water. Adjust the pHwith 10M NaOH, autoclave and store at 4°C. Add 1.38µl of βmercaptoethanol (50mM) just before use.
- Phenol: Cholorform: Isoamulalcohol (24:23:1)

Procedure:

- 1. 1g tissue ground in liquid nitrogen was homogenized in 2ml extraction buffer + 2ml Ph:Chl:IAA. {The sample was homogenized using power operated mini grinder (the steel grinder part was pre-cooled in liquid nitrogen) that perfectly fits in to the falcon tube. It was necessary to maintain frozen conditions throughout the extraction to enhance the quality of the target RNA. }.
- 2. The sample was centrifuged for 10 min at 10,000rpm (at 0-2 °C).
- 3. To the Supernatant, Ph:Chl:IAA (equal volumes in 1:1 ratio) was added and the RNA was precipitated overnight in -20.
- 4. The next day the sample was centrifuged for 20 minutes at 10,000rpm (at 0-2 °C) and the pellet and was dissolved in Deionized water (Volume based on required concentration).
- RNA was stored at -80°C. The quality of RNA was confirmed by using BIO-RAD Smartspec[™] plus Spectrophotmeter and also by Formaldhyde agarose gel electrophoresis (Sambrook et al, 1989).

cDNA LIBRARY CONSTRUCTION

The freeze dried leaves of D. alata L genotypes, Tda 95/00328, resistant to the FGS strain of C. gloeosporioides but susceptible to the SGG strain and TDa 92-2, susceptible to the FGS and SGG strains of *C.gloeosporioides* were obtained from IITA, Ibadon, Nigeria. Leaves were ground in liquid nitrogen and total RNA was isolated using the standardized protocol. Total RNA thus isolated was used for the construction of cDNA library using The Creator smart cDNA library construction kit (BD Biosciences Clonetech). First strand cDNA was synthesized using SMART IV oligonucleotide followed by long distance PCR amplification to generate high yields of full-length ds cDNAs (~400 to >4000 bp) followed by Sfi I digestion and column fractionation. The cDNA fractions that match the desired size distribution (1-4kb) were selected. The Sfi I – digested cDNA was ligated to the Sfi I digested dephosphorylated pDNR-LIB Vector (Clonetech) and transformed into DH10B T1 Phase resistant bacterial cells. The chloramphenicol resistant colonies were picked and archived in 96 well plates. For preliminary round of sequencing, about 100 colonies from each library (resistant and susceptible) were randomly selected and subjected to single pass sequencing (Agencourt Biosciences).

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FIGURE 2. Intact yam rRNA samples using current protocol.

RESULTS AND DISCUSSION

The quantity of total RNA is between 250 to 500μ g from 1g of yam leaf tissue. The 18S and 28S ribosomal RNA bands are clearly visible in the intact leaf RNA samples Dm and Bm of yam (Fig. 2) and the quality reading on spectrophotometer were presented in the Table 1.

Following quality check of the sequences, the pure quality sequences were checked for homology to sequences in GenBank using BLAST similarity search tool. Data obtained from the BLAST analysis of 100 clones from each resistant (Dm) and susceptible (Bm) accessions were compiled and interpreted with respect to the hits identified in other plant species (Table 2 and 3).

This preliminary data describes the initial efforts to develop tools to annotate EST's for anthracnose disease resistance genes by constructing good quality cDNA libraries for different accessions of *D.alata*. From each cDNA library 6000 colonies were arrayed into 96 well plates. A total of 100 clones randomly selected each from two

Sample	$ng/\mu L$	A260	A280	A260	A260	Constant	Cursor	Cursor	340
ID				A280	A230		Pos	Abs	raw
Bm	257.6	6.438	2.997	2.15	1.5	40	230	4.287	0.082
Dm	309.6	7.741	3.646	2.12	1.15	40	230	6.703	0.022

TABLE 1. Spectrophotometer readings of quality RNA samples from yam genotypes.

distinct libraries namely Dm and Bm. Of the 100 cDNA clones from each yam genotype, 10 yielded no sequence and an additional 9 produced sequences of less than 100 bp and these were not used for sequence analysis. The average length of the remaining sequences was 762 bp.

Based on top Blast hits in plants, in yam type Bm, out of 100 sequences, 48 were distinct gave >400bp and were showing functional similarities. In Yam type Dm, out of 100 sequenced clones 48 were distinct, gave >400bp and 22 were duplicates of yam type 1 were observed. The genes putatively identified are shown in Table 2 and 3. The blast hits identified in different crops showed 88-100% identity and, in general, the homology of the insert sequence to the blast hit is about 400-500bp out of 700-800 bp length aligned. The genes (ESTs) identified based on sequence similarity are involved in various putative functions such as gene or protein expression, protein binding, ripening, cell wall and stress response, defense, photosynthesis, photoperiodic flowering response, cell division and proliferation, nodulation, and secondary metabolism etc. and some of them could not be classified into any of these categories.

The numbers of hits showing stress/defense related function were comparatively more in resistant genotype when compared to susceptible genotype (Satya et al, 2007). Of the distinct sequences there are sequences similar to unknown protein and unknown mRNA (1-2%) not presented here. The information on hits to clone sequences (10%) in different crop species and the top blast hits to mitochondrial genes and genes encoding for ribosomal protein genes (20%) were not listed in the table. By sequencing a large number of cDNAs, we can selectively avoid the clones that represent ribosomal and mitochondrial genes, and choose clones that represent genes that we wish to examine. This is a significant improvement compared to previous efforts where sequences coding for ribosomal proteins were predominant in the libraries. This achievement is attributed to quality RNA isolation.

CONCLUSION

Two cDNA libraries for yam, one each for resistant and susceptible genotypes, were constructed for the purpose of identifying clones that are differentially expressed in these two genotypes. Many new genes have been identified that can be useful for future studies. The sequences may also be a source of single-nucleotide polymorphisms or simple sequence repeats for molecular marker development.

Preliminary analysis of 200 clones revealed homologies to known genes in several related and distant plant species. Though the numbers of hits were comparatively more in resistant genotype compared to susceptible genotype, not much distinct differences were observed between the functional hits to sequences of these two genotypes.

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Clone ID	NCBI Definition line for	Crops in which hits were identified
	Putative function of the	
	Blast hits	
Dm 3	mRNA, complete cds;	AC183495.1(Cabbage);
		gb DQ903665.1 (Turnip);
	: DN4 1	dbj AP008209.1 (R1ce)
Dm 4	genomic DNA, chromosome	$db_{j} AP008207.1 ; db_{j} AP001633.2 ,$
Dm 7 52	1,10 Matallathianain lika protain	DO202205 1(sage nalm);
Din 7, 52	(MET grip24 MWMT3)	DQ202303.1 (sago paini); A $I236013.1$ (A frican oil paim);
	(WE1,gmp24,WWWW115)	AJ237000 1(grape):
	mkna, complete	AV857033 1(Cotton):
		$\Delta F_{268393} 1(Banana)$
Dm 8	Solanum lyconersicum	dbi AP009293 1 (brinial):
DIN 0	genomic DNA chromosome	NM_001073500 1
	8 clone: C08HBa0323O07	AP008218 1(Rice)
	complete sequence:Orvza	
	sativa (japonica cultivar-	
	group) genomic DNA.	
	chromosome 12	
Dm 11,	chloroplast, complete	DQ887676.1(Drimys)
32, 69, 59	genome	AJ627251.1(Nymphaea alba);
		AY916449.1(Phalaenopsis
		aphrodite); DQ899947.1 (tulip)
Dm 12, 45	chloroplast mRNA for Tic62	AY437888.1(Sheperd's purse);
	protein;IbJ8 mRNA for JA-	AJ344551.2(Pea);
	domain, complete cds;	DQ499754.2(Potato);
	SrGLU5 mRNA for beta-1,3-	AB246796.1(Sweet potato);
	glucanase, complete cds	AB242267.2(Sesbania);
		AB210846.1(Lemna)
Dm 15	Ribulose-bisphosphate	NM_123204(Arabidopsis).3;
	carboxylase (AT5G38430)	V00458.1(Soybean); AY143814.1;
	mRNA, complete cds;	AY142543.1; AY065026.1
Dm 18	aci-reductone dioxygenase-	DQ244304.1; AY103746.1(Maize);
	like protein (ARD) mRNA,	CT831853.1, NM 001055581.1,
	complete cds	AY955841.1(Rice);
		AB025597.1(Barley)
Dm 21	Oryza sativa (japonica	dbj AP008208.1
	cultivar-group) genomic	
	DNA, chromosome 2	
Dm 24	mRNA sequence complete	NM 001032532.1
	cds	_
		1

TABLE 2. Blast hits from cDNA library of Yam accession Dm

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RNA ISOLATION PROTOCOL

Dm 30	Oryza sativa microsatellite	AY023257.1
	MRG5582 containing	
	(GGA)X13. genomic	
Dm 34	Medicago truncatula clone	AC136839.20
-	mth2-13n2, complete	
	sequence	
Dm 42	Full-length cDNA Complete	CR936947.2 , emb
	sequence from clone	CR931731.1 (Medicago);
	-	BX821860.1(Arabidopsis)
Dm 48	Glycine max mRNA for	gi 109940718 emb AM158274.1
	asparagine	
Dm 53	cDNA clone:full insert,	NM_001066955.1;
	mRNA sequence,complete	AK070897.1(Rice);
		AY107920.1(Maize)
Dm 54	Zea mays cultivar Mo17	AY664418.1
	locus 9008, complete	
Dm 58	Sequence of BAC E1511	AC006577 2: AB077139 1
Din 56	from Arabidonsis thaliana	AC000377.2, AD077137.1
	chromosome 1; Prunus	
	persica DNA, microsatellite	
	marker MA035a	
Dm 59	Croomia pauciflora large	DQ629350.1(Nuttall);
	subunit ribosomal RNA gene,	DQ629349.1(Yam)
	partial; Dioscorea sp. Qiu	
	ribosomal RNA gene nartial	
Dm 61.	Drosophila melanogaster	AE014296 4(Drosophila):
Dm 80	chromosome 3L, complete	AJ831399.1(Santalum)
D III 00	sequence: Santalum	
	austrocaledonicum	
	microsatellite DNA clone	
	mSaCIRF04	
Dm 64	Lycopersicon esculentum	AF411804 1
Din 01	BAC clone Clemson ID	
	47113 complete	
Dm 65	histidine ammonia-lyase-like	EF051316.1(Gymnadenia):
D III OU	mRNA complete	BT012683 1(Tomato):
		AC157536 31(Medicago)
Dm 70	Oryza satiya (japonica	AP008209.1
2 , 0	cultivar-group) genomic	
	DNA, chromosome 3	
Dm 79	Zea mays clone 92533	DO245928.1
2	mRNA sequence	
Dm 88	Brassica rapa subsp.	AC189444.1
2	nekinensis clone	
	KBrB070123 complete	
1	KDID0/0323, complete	1

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Dm 89	Sorghum bicolor clone SB_BBc0020007, complete sequence	AC169375.4
Dm 91	Arabidopsis thaliana unknown protein (AT2G19830) mRNA, complete; Lycopersicon esculentum clone 134156F, mRNA sequence; Zea mays clone EK07D2310A10. c mRNA sequence	NM_127541.2; BT013152.1(Tomato); BT017005.1(Maize)

TABLE 3. Blast hits from cDNA library of Yam accession Bm

Clone ID	NCBI Definition line for Putative function of the Blast hits	Crops in which hits were identified
Bm 2, 13, 83	Metallothionein-like protein (MET) mRNA, complete	DQ202305.1(Sago palm), AJ236913.1(African oil palm), Grape, cotton, citrus, musa and rice
Bm 3, 5, 16, 17, 22, 23, 25, 26, 30, 45, 53, 66, 69, 76, 88, 91, 41, 52, 34, 38, 57, 73	Mitochondrial, chloroplast DNA, complete sequence; ribosomal RNA gene partial	DQ887676.1(Drimys granadensis); AJ627251.1(Lotus); AB240139.1(Tobacco); DQ629360.1(Dicentra Sp.); DQ340440.1(Pacific Dogwood), DQ923117.1(Heavenly Bamboo); AF205123.1; DQ629349.1, DQ629457.1(yam); DQ629350.1(Nuttall)
Bm 4	cDNA clone:OSIGCFA011A01, full insert sequence;	CT829335.1(Rice) ; AY224463.1(Rice)
Bm 12	mRNA for Mob1-like protein (mob1-B) complete cds	AY437888.1(shepherd's purse), AM161645.1(alfalfa)
Bm 20, 37	LpLHY H2 mRNA for LHY homologue2, complete	AB210846.1(Duckweed), DQ499754.2(Potato)
Bm 31	unknown protein (AT2G46100) mRNA, complete	NM_130173.3(Arabidopsis), BT012819.1(tomato)

RNA ISOLATION PROTOCOL

Bm 35,	lipid transfer protein mRNA,	EF031153.1(Stevia);
63	complete cds	AY395741.1(Summer grape)
Bm 43	beta-1,3-glucanase, complete,cds	DQ499754.2(Potato); AB246796.1(Sweetpotato);
		AB242267.2(Sesbania)
Bm 47	Oryza sativa (japonica cultivar-group) genomic DNA, chromosome 1	AP008207.1(Rice)
Bm 48	aspartic proteinase 4, complete cds	CT829760.1(Rice); AB045894.1(Nepenthes); NM_001049320.1(Rice); AY103982.1(Rice)
Bm 55	putative terpene synthase, complete cds	AK227599.1(Arabidopsis); NM 001054333.1(Rice)
Bm 59	Full-length cDNA Complete sequence from clone	BX817199.1(Arabidopsis)
Bm 60	Brassica rapa subsp. pekinensis clone KBrB002G19, complete; Capsella bursa-pastoris ecotype CZ96 microsatellite ATCP70189	AC189190.1(Chinese cabbage); DQ144500.1(sheperd's purse)
Bm 32, 61, 62, 64	cDNA clone: full insert sequence;mRNA, complete sequence	AC137065.26(alfalfa), DQ244538.1, DQ245784.1, DQ244442.1(maize); CT830019.1(Rice); AK069033.1, CT829171.1, CT830462.1(Rice); AP006116.1(Lotus); BT014284.1(Tomato); AY085715.1(Arabidopsis);
Bm 75	Lotus japonicus genomic DNA, chromosome 3, clone: LjT13M14	AP004531.1(Lotus)
Bm 17, 39, 80	Nicotiana tabacum chloroplast pigment-binding protein CP29 (Lhcb4); Panax ginseng cab mRNA for chlorophyll a/b binding protein; Nicotiana tabacum chlorophyll a/b binding protein mRNA, complete	AB236867.1(Ginseng); DQ676843.1, AY219853.1(Tobacco); CT829715.1(Rice)
Bm 82	ubiquitin conjugating enzyme (UBC4), complete cds	L29077.1(Peas); CT833517.1(Rice); AY086109.1(Arabidopsis)
Bm 92	Populus trichocarpa clone Pop1-21114, complete sequence	AC182669.2(Populus)
Bm 94	Glycine max mRNA for asparagine synthetase, type III (sas3 gene)	AM158274.1(Soybean)

Therefore this project is revised to continue cDNA library construction from challenged leaf tissues of these two genotypes besides including a third genotype resistant to SGG strain to identify the candidate genes to anthracnose resistance. The ESTs generated in this study also provide a good tool for more studies to understand the resistant and susceptible interactions of yam anthracnose.

Analysis of sequences from recently completed revised yam genomics project will generate more ESTs for differential expression analysis for the purpose of identifying candidate genes for anthracnose resistance, marker development and further yam QTL mapping studies.

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S.Narina., A.Mohamed., R.Asiedu and HD Mignouna were involved in the design of the study, carried out the molecular experiments, analysed the data and drafted the paper. R. Asiedu supplied the material and drafted the paper. All authors read and approved the final manuscript.

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Management and Social Indicators of Soil Carbon Storage in a Residential Ecosystem, Midlothian, VA

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ABSTRACT

Soil carbon storage - defined here as carbon mass per unit ground area - is an important ecosystem service, sequestering carbon that might otherwise exist in atmospheric CO₂. Significant attention has focused on the effects that humans have on carbon cycling, but little is known about how human behaviors and attitudes relate to lawn carbon storage. The objectives of this study were to conduct household surveys in concert with soil carbon sampling in a 10-yr-old exurban neighborhood near Richmond, Virginia to quantify differences in soil carbon storage between residential lawns and mixed pinehardwood forest fragments, and to determine how lawn management and environmental attitudes relate to soil carbon storage. Lawns stored significantly less carbon than forest fragments in the top 10 cm of soils. A significant negative relationship was observed between watering and fertilizer frequency and soil carbon storage, but the goodness-of-fit was sensitive to intra-lawn variability in soil carbon mass. Survey respondents that claimed to be environmentalists stored significantly more carbon and spent one hour less per week managing their lawns, suggesting that environmental attitudes may affect how households manage their lawns and, in turn, the quantity of soil carbon stored in residential soils.

Key words: Soil carbon, carbon sequestration, lawn, human-dominated, residential, management

INTRODUCTION

Deforestation during land-use conversion is a principal determinant of the carbon balance of the conterminous United States, prompting C emissions of 12 Tg yr⁻¹ from 1990-2004 (Woodbury et al. 2007). A substantial fraction of C emissions to the atmosphere in the U.S. is attributed to land-use conversion from forest to residential ecosystems, which increased from 2.5 to 3.1% from 1990-2000 (Nowak et al. 2005). Although disturbance during land-use conversion may cause an initial precipitous decline in soil carbon storage, defined here as soil carbon mass per unit area, post-

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conversion management of residential lawns is hypothesized to prompt recovery of soil carbon storage that in some cases exceeds that of undisturbed forests (Pouyat et al. 2006, Pouyat et al. 2007, Pickett et al. 2008). The proposed mechanism for higher soil carbon in highly productive residential lawn 'ecosystems' is the accumulation of grass derived organic matter (Milesi et al. 2005). Nowhere in the U.S. is land-use conversion from forest to residential habitat more prominent than in the southeastern Piedmont region, which is now experiencing net deforestation for the first time in nearly a century as the region rapidly urbanizes (Polsky et al. 2000).

The amount of soil C stored in residential ecosystems following land-use conversion from forests is determined by the balance of carbon inputs and outputs over time, and carbon storage prior to land-use conversion (Pickett et al. 2008). Humans substantially alter the balance of carbon inputs and outputs through management of residential lawns (Baker et al. 2007). Management regimes that augment lawn net primary production (i.e., growth) and retain grass clippings increase carbon inputs to residential soils (Heckman et al. 2000, Kopp and Guillard 2002, Qian et al. 2003). Effects of common lawn management practices on soil organic matter decomposition and, consequently, carbon outputs via microbial respiration are less certain, but soil disturbance generally causes carbon losses (Kaye et al. 2005).

Despite its probable importance, lawn management at the household scale plays an uncertain role in determining soil carbon storage in residential ecosystems, especially in the understudied, urbanizing Piedmont region of the southeastern U.S. (Kaye et al. 2006, Pataki et al. 2006, Pickett et al. 2008). The Virginia Piedmont is a model location for examining the presently poorly understood consequences of land-use transformation on carbon cycling because urban growth is equal to or greater than that of many areas in the region (Rogers and McCarty 2000). Numerous studies conducted in other geographic regions have quantified carbon storage in soils and other pools within human-dominated ecosystems (Jo and McPherson 1995, Koerner and Klopatek 2002, Nowak and Crane 2002, Kaye et al. 2004, Ziska et al. 2004, Kaye et al. 2005, Milesi et al. 2005, Golubiewski 2006, Groffman et al. 2006, Pouyat et al. 2006). Only a few studies have taken soil carbon analyses a step further by empirically examining soil carbon storage across a range of lawn management intensities (Qian and Follett 2002), or in relation to social indicators that, in human-dominated ecosystems, may be robust integrated predictors of soil carbon properties (Pataki et al. 2006, Pickett et al. 2008). Additionally, educational campaigns that aim to reduce household carbon footprints require knowledge of how residential soil carbon storage relates to human behavior and attitudes. For example, carbon footprint models that predict the carbon signature of households from human behavior are proposed tools for educational outreach and behavior interventions (Dilling et al. 2003, Pataki et al. 2006, Dilling 2007b).

Here, household surveys of lawn management behavior and environmental attitudes were conducted, and soil carbon mass (to 10 cm depth) quantified in lawns and forest fragments of a 10-yr-old exurban neighborhood near Richmond, Virginia to: 1) determine whether soil carbon storage differed between forests and lawns; and 2) identify lawn management, physical, and social indicators of soil carbon mass in a residential ecosystem. An important secondary objective of this work is to examine the feasibility of developing simple models for predicting soil C storage from lawn management practices. The study focuses on an understudied system that is increasingly typical of the Piedmont region of southeastern U.S. in which a planted pine

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forest with hardwood representation is converted to a residential ecosystem (Polsky et al. 2000). The Piedmont comprises a distinct physiographic region of the U.S. with unique soils, climate, and socio-demographic features, suggesting that soil carbon patterns may differ from those reported by prior studies conducted in other geographic areas. Additionally, this study is among the first to link human management behavior and attitudes to soil carbon storage following land-use conversion from forest to residential lawn.

METHODS

Study location

The study was conducted in an upper-middle class exurban neighborhood located 30 km west of Richmond in Midlothian, VA (zip code 23112). Single family homes $>300 \text{ m}^2$ were built in the middle to late 1990s on residential parcels ranging from 0.25 to 0.5 ha. Human population density was 6440 km² with a median household size and annual income of 3.26 and \$85,000 USD, respectively, in the year 2000 (United States Census Bureau 2000).

The dominant ecosystem prior to land-use conversion to a suburban residential neighborhood, as indicated by the surrounding forest fragments, was a mature forest typical of the region comprised of planted loblolly pine (*Pinus taeda* L.) and volunteer hardwood competition including oak (*Quercus*), hickory (*Carya*), and maple (*Acer*) genera. Homes within the suburban neighborhood are distributed throughout fragmented forest remnants, which are preserved in common space that is used for recreation (Figure 1). Residential soils are a Creedmoor fine sandy loam on slopes of 0-12 % (USDA 2009). Mean January and July air temperatures are 6.1°C and 25.6°C, respectively, with mean annual air temperatures of 14.3°C and mean annual precipitation of 1115 mm (NOAA 2009).

Experimental design and household surveys

The experimental design coupled household surveys of lawn management and environmental attitudes with lawn soil bulk density and carbon sampling in a single neighborhood. To examine impacts of forest conversion to lawns, soils were sampled in residential lawns and adjacent forest fragments distributed throughout the neighborhood. The examination of a single, exurban neighborhood minimized variation in soil properties caused by differences in time since land-use conversion, climate, soil type, parcel size, and household affluence, all of which may constrain soil carbon percent and mass (Pickett et al. 2008).

Sixty household surveys of lawn management behavior and environmental attitudes randomly distributed within the study neighborhood in the autumn of 2008 yielded 33 respondents. Surveys inquired about: a) the application, irrespective of frequency, of soil aeration, lawn clipping retention, raking of detritus, mulch, chemical weed control, chemical pesticides, and seeding; and b) the frequency of mowing, watering, and bagged fertilizer application during the autumn, spring, and summer months. Two additional questions asked respondents to describe their alignment with environmental issues and how many hours per week they work in their lawns during the growing season.



FIGURE 1. Aerial view of the residential neighborhood examined for soil properties in Midlothian, Virginia, 30 km outside of Richmond. The inset ruler is 100 m.

Soil bulk density and carbon percent and mass

The O horizon and mineral soil carbon were sampled together to a 10 cm depth during the dormant season of 2008-2009 in 33 residential lawns for which management practices were determined via household surveys, and in 24 randomly selected locations within three large, contiguous forest fragments encompassing the residential areas. Sampling was limited to the top 10 cm since this carbon-rich soil surface pool is likely more sensitive to disturbance, including land-use transformation from forest to lawn, and also is more responsive to subsequent management (Pouyat et al. 2006). To minimize potential bias from recently deposited detritus (e.g., leaf litter, woody debris), fresh litter (O, horizon) was removed from the surface prior to using a metal corer (3.0 cm diameter) to extract soil from each sampling location. It is important to note that because of composite sampling of O and mineral soils, physiochemical properties reported are a combination of both horizons. In each residential lawn, soil subsamples were collected at three locations 10 m apart along a transect running parallel to each house. As a proxy for local microclimate, aspect and slope were recorded for each soil collection location. Forest soils were collected from locations with no visible disturbances that were > 20 m interior to the forest boundary and within the common area of the neighborhood. Soils were stored at -20°C until processed.

In the laboratory, soils were sieved through a 2 mm mesh screen, remaining roots

SOIL CARBON STORAGE IN RESIDENTIAL ECOSYSTEMS

Parameter	Lawn	Forest fragment
Bulk density (g cm ⁻²)	0.92 (0.035)*	0.41 (0.037)*
Percent carbon	3.85 (0.27)*	16.54 (1.04)*
Carbon mass (kg m ⁻²)	3.30 (1.45)*	5.50 (1.24)*

TABLE 1. Summary of soil properties (to 10 cm depth) and statistics for lawns (n = 33 lawns) and forest fragment soils (n = 24) in Midlothian, VA.

Mean ± 1 standard error, **P* < 0.0001

were removed, and root-free soil was dried in an oven at 60° C to a constant mass and weighed to obtain bulk density. A portion (~ 10 g) of each soil subsample was ground with a mortar and pestle, weighed, and loss on ignition (450°C for 12 hrs) was used to calculate carbon content assuming a 0.58 C fraction (Pouyat et al. 2002). Soil bulk densities were multiplied by the percent carbon of respective subsamples to calculate soil carbon mass.

Statistical analysis

A stepwise model selection procedure was used to determine which lawn management and climate proxy parameters (i.e., aspect and slope) correlate with spatial (inter-lawn) variation in soil carbon mass. Soil subsamples taken from the same lawn were averaged for the analysis. Separate modeling analyses were conducted on lawns with coefficients of variation < 0.25 for soil carbon mass and on all lawns to determine how within-lawn variation affected model explanatory power. Lawn management and climate proxy parameters were retained in the regression model when alpha \leq 0.15, the default for the stepwise procedure in SAS statistical software (SAS Institute, Cary, NC, USA) and a commonly accepted alpha for regression modeling analysis (Montgomery et al. 2001).

Two-tailed t-tests were used to compare mean bulk density, soil carbon percent, and soil carbon mass between lawn and forest soils. A Wilk-Shapiro Normalilty test revealed that data were normally distributed and required no pre-analysis transformation. ANOVA with LSD was used to assess differences in soil carbon mass among self-ascribed environmental behaviors. All statistical analyses were conducted using SAS v. 9.1.

RESULTS

Lawn and forest soil properties

All residential lawn soil properties surveyed differed significantly from those of forest soils (P < 0.0001, Table 1). Forest soil bulk density in the top 10 cm was less than half of that observed in lawns, but soil carbon percent in forest soils was over 4 times greater than that of lawns (P < 0.0001, Table 1). This resulted in 67 % greater soil carbon storage in the top 10 cm of forest soils (Table 1).

1	awn Management Practice or Physical Indicator	Effect on Soil Carbon Mass	Partial r^2	Р
1.	Watering frequency during growing season	ļ	0.18	0.06
2.	Southeast facing orientation of lawn	Ļ	0.11	0.11
3.	Fertilization frequency during	Ţ	0.10	0.11

TABLE 2. Relationships between lawn management practices and orientation, and soil carbon mass (to a depth of 10 cm) in an exurban neighborhood near Richmond, VA. Parameters were selected using a stepwise model procedure ($\alpha = 0.15$).

Lawn management, orientation and soil properties

Stepwise model selection indicated that soil carbon mass was significantly correlated with the frequency of common lawn management practices, watering (P = 0.06) and fertilization (P = 0.11), and with lawn cardinal orientation (P = 0.11). Higher frequencies of lawn watering and fertilization during the growing season corresponded with lower soil carbon mass in the top 10 cm (Table 2). Lawns that were oriented toward the southeast also had lower soil carbon mass than those facing northwest.

Based on these modeling results, an integrated management and orientation index was developed for predicting inter-lawn variation in soil carbon mass to a 10 cm depth. Discrete points were assigned to lawns with higher watering and fertilization frequencies during the growing season, and to those more closely oriented in the southeastern facing direction (Table 3). Thus, a high index value indicates greater lawn management intensity and an orientation toward a putatively dryer, warmer southeastern face. This index, when fitted against soil carbon mass using an exponential decay function, explained 57 % (P = 0.0006) and 18 % (P = 0.05) of the variation in soil carbon mass among lawns with low within-site variation (C.V. < 0.25) and among all lawns, respectively. Soil carbon mass in the top 10 cm exhibited a rapid decline from 4.5 kg m² in lawns with low indexes to a near asymptotic low of 2.8 kg m² in lawns with moderate to high indexes (Figure 2).

Environmental attitudes and soil carbon mass

Self-ascribed alignment with environmental issues was a moderate indicator of soil carbon mass (Figure 3). Survey respondents who claimed to be strong environmentalists had lawns with significantly greater soil carbon mass by 0.8 kg m⁻² and they spent one hour less per week on lawn work than those who said that they agree with environmentalists on most issues. Statistical differences among other respondent categories were not significant (P > 0.1). Only one respondent claimed to not be an environmentalist at all and, because of insufficient replication, was excluded from statistical analysis.

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TABLE 3. Point assignments used to calculate lawn management and orientation indexes for individual lawns. Parameters were selected for the index using a stepwise modeling procedure when $\alpha < 0.15$ (see Table 2). Lawn indexes were calculated for each surveyed household by summing points associated with each parameter.

Parameter	Point assignment
Watering frequency during growing season	Never = 0; Monthly = 1; Weekly = 2; Daily = 3
Fertilization frequency during growing season	Equals number of fertilizer applications following manufacturer specification (0 to 4)
Cardinal orientation facing lawn	Northwest $(270-360^{\circ}) = 0$; Northeast $(0-90^{\circ})$, Southwest $(180-270^{\circ}) = 1$; Southeast $(90-180^{\circ}) = 2$
Total possible points	9

DISCUSSION

Results from this study show that land-use conversion from forest to lawn significantly reduced carbon storage in the top 10 cm of soil. Forest soil carbon storage reported in this study of 5.5 kg m⁻² in the top 10 cm is similar to 5 kg m⁻² reported for a suburban forest in Baltimore (Pouyat et al. 2002). The mean residential lawn soil carbon mass of 3.3 kg m⁻² in the top 10 cm is somewhat lower than that of other urban and suburban lawns of the eastern U.S. sampled to a 15 cm depth (Pouyat et al. 2002). Pouyat et al. (2009) observed comparable carbon storage in soils of >40-yr-old residential lawns and remnant urban forests of Baltimore city. The present study may have revealed lower soil carbon storage in lawns because land-use conversion from forest to exurban lawns was relatively recent (10 yrs), while older urban lawns of Baltimore have had substantially more time to accumulate carbon. Accumulation of soil carbon occurred for decades following land-use conversion from native habitat to residential lawns or golf course greens located in the arid western U.S. (Qian et al. 2003, Golubiewski 2006).

Results from the present study also indicate that lawn soil carbon storage declined with increasing management intensity. Lawn soil carbon storage in this study was negatively correlated with increased fertilization and watering frequency, and with a more southeastern facing lawn orientation. Empirical studies conducted in golf courses indicate mixed effects of fertilization on soil carbon storage, reporting either no effect (Qian and Follett 2002) or a positive effect (Higby and Bell 1999) of fertilization on soil carbon storage. Modeling studies uniformly predict a net increase in soil carbon storage with management intensification (Bandaranayake et al. 2003, Qian et al. 2003, Milesi et al. 2005). Findings from these empirical and modeling studies provide important quantitative assessments of how management behavior might affect soil



FIGURE 2. Lawn soil carbon mass (to 10 cm depth) in relation to integrated management intensity and lawn orientation indexes (see text and Table 3 for details), and in comparison to soil carbon mass of surrounding forest fragments. A higher index indicates greater lawn management intensity and/or a more southeastern facing orientation. Regression analysis was conducted using mean lawn soil carbon mass values with coefficient of variations (C.V.) < 0.25 (black filled circles; n = 21). Means with C.V. > 0.25 are also shown (black X's; n = 12). Gray-shaded area is the 95 % confidence interval of forest soil carbon mass (n = 24).

carbon storage; however, results from the present study suggest that residential ecosystems, which encompass a range of complex management behaviors, may not uniformly respond to common management practices in the same way.

Declining soil carbon storage with higher fertilization and watering frequency will occur if these parameters cause soil carbon inputs to decrease or carbon outputs to increase. Soil carbon storage decline is unlikely to be caused by a reduction in carbon inputs with fertilizer and water amendments because these supplements typically increase lawn primary production (Higby and Bell 1999, Qian et al. 2003, Milesi et al. 2005). Contrastingly, water and fertilizer amendments may stimulate microbial decomposition of soil organic matter, thereby increasing carbon losses from soils (Kaye et al. 2005, Rodriguez et al. 2005). Although the present study did not detect relationships between aeration and soil carbon storage, tilling and aerating stimulated soil organic matter decomposition in agricultural soils (Reicosky et al. 1997, Kandeler et al. 1999, Paustian et al. 2000). High management intensity in residential ecosystems



FIGURE 3. Soil carbon mass (10 cm depth) in relation to household views on environmental issues and to hours invested in yard work during the growing season. Survey respondents (n = 33) were asked: "How would you describe yourself with respect to environmental issues?" and "How many hours per week, during the growing season, do you spend caring for your lawn?" Letters indicate significant differences among means ($\alpha = 0.05$).

is a putative cause for elevated microbial activity, and consequently decomposition rates, relative to surrounding natural ecosystems (Green and Oleksyszyn 2002, Koerner and Klopatek 2002, Kaye et al. 2005). In the present study, the mechanistic cause of declining soil carbon with increasing management intensity is unknown, but may be due to enhanced microbial activity. No other management behaviors (e.g., leaving

clippings onsite) were significantly correlated with soil carbon storage in the present study and autocorrelation among management behaviors was not detected.

Soil carbon inputs and outputs are also constrained by microclimatic conditions (Bandaranayake et al. 2003, Milesi et al. 2005), which likely varied according to lawn orientation in the present study. Lower soil carbon storage in lawns facing southeast may be caused by dryer, warmer microclimates, which could concurrently reduce net primary production and increase temperature-limited rates of microbial decomposition of organic matter in lawns that are well-watered (Wythers et al. 2005, Del Grosso et al. 2008). Additional investigation is required to quantify the balance of carbon inputs and outputs to residential soils. Particularly, quantitative assessments of carbon outputs are needed for residential ecosystems since most studies have investigated the contribution of carbon inputs to soil carbon storage (Kaye et al. 2006).

Findings from the present study provide novel support for the notion that social indicators can be useful, integrated predictors of soil carbon storage in residential ecosystems. Households that claimed to be more supportive of environmental issues stored significantly more carbon in their lawns (Figure 3), possibly because they spent less time managing their lawns in a way that may reduce soil carbon storage. Strong environmentalists, for example, spent less time managing their lawns and, consequently, watered and fertilized less frequently, behaviors negatively correlated with soil carbon storage in the present study. Results from this study are supported by a limited number of reports that show social indicators are robust predictors of ecosystem properties in human-dominated systems. For example, Tratalos et al. (2007) showed that demographic indicators of social status correlate with residential carbon storage rates in the United Kingdom. Other studies examined how lifestyle behavior or social status relate to parameters known to affect carbon storage, including vegetation cover and tree density (Grove et al. 2006) and fertilizer application rates (Robbins et al. 2001). Qualitative and semi-quantitative social indicators are promising predictors of ecosystem function in human-dominated ecosystems and may be important components of future "carbon footprint" models for urban areas; however, substantial additional research is required to determine which social indicators are the best predictors of residential soil carbon storage and to determine whether management attitudes and behaviors are causally linked (Whitford et al. 2001, Pataki et al. 2006, Grimm et al. 2008).

Results from the current study show that a simple model for estimating soil carbon storage in residential ecosystems may hold future promise, but predictive power is presently limited by unexplained spatial variability in soil carbon mass. High withinlawn variation in soil carbon storage limited the detection of strong statistical relationships with management behavior and orientation when all lawns were included in model development. Soil carbon storage was significantly correlated with the integrated index even when all lawns were included in the regression analysis ($P \le$ 0.05), but this caused a substantial decline in the model's explanatory capabilities. It is also important to note that this study is of a single neighborhood and, although this approach best addressed study objectives, results are limited in inference to ecosystems with similar social (e.g., economic) and physical (e.g. soils) dimensions. Despite these limitations, this study suggests that the general approach employed herein could be successfully modified to incorporate additional putative explanatory variables that aid in the development of more robust predictive models.

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CONCLUSION

As residential ecosystems grow in number and area, numerous calls have been made to understand how human behavior modifies important ecosystem functions, such as carbon cycling (Vitousek et al. 1997, Pickett et al. 2005, Pataki et al. 2006, Liu et al. 2007, Pouyat et al. 2007, Grimm et al. 2008). Vitousek et al. (1997) asserted that contemporary ecosystem processes cannot fully be understood without investigating how and why humans interact with surrounding ecosystems.

This study is the first conducted in the Piedmont region of the southeastern U.S. to show that household lawn management is a significant predictor of soil carbon storage in residential ecosystems. Further investigation is warranted to evaluate why lawn management intensification decreased lawn soil carbon storage in the present study, a result that departs from some experimental and modeling studies conducted in other geographic regions. A broader understanding of household effects on carbon cycling in residential ecosystems will have implications for ongoing educational campaigns that seek to modify human behaviors that affect greenhouse gas emissions (Dilling 2007a).

ACKNOWLEDGMENTS

Chris Gough and Eliza Fritz worked jointly to develop methods described in this manuscript. Eliza, a Clover Hill High School student, conducted most of the data collection, soil processing, and preliminary data analysis and summary. Chris performed the final data analysis and manuscript preparation.

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Dear VAS Member:

The VAS Fall Meeting for Undergraduate Research will be held on October 16th at the Science Museum of Virginia. At this meeting, five \$500 research grants will be awarded to undergraduate students to support their research during the 2010-11 academic year. In order to be eligible for a research award, the student(s) must submit a brief research proposal by October 1, 2010 to: Dr. Michael Renfroe Dept. of Biology, MSC 7801 James Madison University Harrisonburg, VA 22807.

The faculty mentor for the project must be a member of the Virginia Academy of Science by the October 1st deadline for submission of the grant application. The students are required to attend the VAS Fall Undergraduate Research Meeting and present a poster outlining their proposed research. Both the research proposal and the poster presentation will be evaluated to determine the recipients of the research awards. The application for the Undergraduate Research Grants is attached and is also available on the Virginia Academy of Science web site, **www.vacadsci.org**. In addition, the recipients of the research awards will present the results of their work at the VAS Annual Meeting in May.

As I indicated above, this year the fall meeting will be held on October 16th at the Science Museum of Virginia, 2500 West Broad Street, Richmond. The poster session will be held in the morning followed by lunch, guest speaker and announcement of the research awards.

We are very excited about this program and hope that you will encourage your undergraduate research students to participate. Also, please pass this information on to other faculty at your institution who sponsor research students and encourage them to become members of the Academy and to participate in this program. Help us to make this a successful program and one that we can expand in the future.

Sincerely, Michael Renfroe, Chairman Fall Meeting Committee

VAS Fall Meeting for Undergraduate Research SCHEDULE OF EVENTS Saturday, October 16, 2010

- 9:00 arrival time 9:00-10:00 poster set-up and coffee hour 10:00-12:00 evaluation of posters (During this time period, each poster will be evaluated by a team of judges. The judges will meet with each student and the student should be prepared to give a brief presentation (no more than 5 minutes) on the proposed research and to answer questions from the judges.) 12:00-1:00 lunch 1:00 - 2:00 Invited Speaker
- 2:00 2:30 announcement of grant recipients
- 2:30 3:00 remove posters and depart

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